Introduction

Root system architecture: insights from Arabidopsis and cereal crops

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Roots are important to plants for a wide variety of processes, including nutrient and water uptake, anchoring and mechanical support, storage functions, and as the major interface between the plant and various biotic and abiotic factors in the soil environment. Understanding the development and architecture of roots holds potential for the exploitation and manipulation of root characteristics to both increase food plant yield and optimize agricultural land use. This theme issue highlights the importance of investigating specific aspects of root architecture in both the model plant Arabidopsis thaliana and (cereal) crops, presents novel insights into elements that are currently hardly addressed and provides new tools and technologies to study various aspects of root system architecture. This introduction gives a broad overview of the importance of the root system and provides a snapshot of the molecular control mechanisms associated with root branching and responses to the environment in A. thaliana and cereal crops.

Keywords: root; Arabidopsis; maize; rice; hormones; environment

1. INTRODUCTION

Plants are undeniably important to humans and indeed, to most life on Earth. The benefits to human-kind provided by plants include food, fuel, fibres, medicines and materials as well as ornamental and leisure uses. It is easy to think of plants purely in terms of the clearly visible, above-ground parts of plants, such as leaves, flowers, fruits and stems; however, this is only half the story. Plants also demonstrate a considerable degree of variability in the less visible underground elements: root systems. Root system architecture (RSA) varies between species, and also within species, subject to genotype and environment [1]. The importance of root systems combined with the inherent difficulty of studying them has led to roots being described as ‘the hidden half’ [2].

Roots are important to plants for a wide variety of processes, including nutrient and water uptake, anchoring and mechanical support. In addition, many plants use roots for storage functions, and some root structures serve as human food sources, including root vegetables (such as carrots, parsnips, turnips and many others) and spices (such as ginger, arrowroot, liquorice and others). Roots serve as the major interface between the plant and various biotic and abiotic factors in the soil environment: by both sensing and responding to environmental cues, roots enable plants to overcome the challenges posed by their sessile status. This is seen in the many ways plants can dramatically alter their root architecture to optimize growth in a large variety of environmental and soil nutrient conditions. Root architecture has traditionally been largely ignored by plant breeders in terms of potential yield increases, and was not a major selection criterion as part of the crop development programmes of the 1960s’ green revolution [3]. Understanding the development and architecture of roots thus holds potential for the exploitation and manipulation of root characteristics to both increase plant yield and optimize agricultural land use [4].

2. WHY BOTHER?

With the global population projected by the UN to rise to over 9 billion by 2050, improvement of crops is becoming an increasingly pressing issue. Crop plants, particularly cereals, are a vitally important food supply, both directly and through use in animal feed: an estimated 75 per cent of the human energy demand is fulfilled by cereal starch [5]. The ‘green revolution’ of the 1960s and 1970s helped agriculture to meet the food demands of a rapidly growing global population, through the creation of dwarfed cereal cultivars, better irrigation and the application of fertilizer. This resulted in a doubling of cereal yields in developing countries between 1960 and 1990 [6]. Fifty years on, and the benefits of this revolution appear to have peaked: the global population since the 1960s has more than doubled, fresh water shortages hinder large-scale irrigation, and further fertilizer application would be damaging in both crop...
yield and environmental terms (as well as being economi-
cally unfeasible in many third-world countries without
considerable subsidies) [7]. The demand for biofuels (in such circumstances where the production
of such fuels directly competes with arable land) can
also reduce the area of arable land that can be dedi-
cated to food production [8]. The challenge for the
‘second green revolution’ is to solve the current and
future obstacles to maintaining food security through
higher crop yields.

Drought is the most common environmental con-
dition impeding crop growth and productivity [9];
furthermore, currently 70 per cent of the world’s
fresh water demand is for agricultural use [10].
Drought is a threat which is likely to become more
pressing with the predicted future global increase in
temperature [11]. Improved access to deep soil
water, reducing the need for irrigation, is one potential
benefit that could be achieved by exploitation of RSA.
Already, modelling techniques suggest that rising yield
trends in the US Corn Belt can be attributed to the
selective breeding of maize with increasingly deeper,
more vertically orientated root architectures, leading
to more efficient soil water use and increased crop
density [12]. Various studies have also evaluated
breeding of root phenotypes for increased phosphorus
acquisition and carbon sequestration [13,14], and the
latter is also addressed in this issue by Kell [15].

Application of inorganic fertilizer, containing min-
eral nutrients such as nitrogen, phosphorus and
potassium, has traditionally been used to increase yield
in cereals and other crop plants. However, these fertili-
zers are expensive, often have negative impacts on soil
and waterway ecosystems, and fertilizers (particularly
nitrogen fertilizers) require large amounts of energy—
mainly derived from fossil fuels—to produce [16]. In
addition, global reserves of nutrients such as phos-
phorus are limited, with supplies projected to be
depleted in 50–100 years time [17]; and nutrient acqui-
sition by roots is rather inefficient, with acquisition rates
of approximately 33 per cent for nitrogen and 20 per
cent for phosphorus [18,19]. Climate change adds
further pressure to develop crops that can cope with
higher temperatures, increased water salinity and
denser pest loads [8]. Root system exploitation and
modification in crops may enable plants to make more
efficient use of existing soil nutrients and increase
stress tolerance, improving yields while decreasing the
need for heavy fertilizer application.

Many of the advances in understanding RSA to
date have come from studies on the model plant,
Arabidopsis thaliana (referred to as Arabidopsis). For
example, the description of the cellular structure of the
Arabidopsis root by Dolan et al. [20] provided the
basis for future developmental and genetic work.
Because RSA can have major influences on both plant
yield and the ability of a plant to be grown in ‘marginal’
environments, understanding RSA in cereals and bio-
fuel plants is a necessity. RSA in cereal plants has been
a comparatively little-studied area thus far, but newer
studies are starting to address this neglected area—the
first step in translating research on the molecular level
into the tangible benefits of exploiting RSA to improve
crop biomass and yield.

3. ROOT SYSTEM ARCHITECTURE
RSA on a macroscale describes the organization of the
primary and lateral roots, and also accessory roots
(including other root types found in cereals) where
they are present, and is a key determinant of nutrient-
and water-use efficiency in plants. On a microscale,
this includes root hairs that increase the surface area,
aiding with uptake of water and nutrients [21–25]. In
both dicots and monocots, adventitious roots are also
sometimes formed post-embryonically at the root–
shoot junction, for example to explore and exploit the
phosphorus-rich upper soil strata (figure 1a,b). Here,
we will mainly describe root branching and we refer to
recent reviews on (molecular) control mechanisms of
primary root [26,27] and root hair development [25,28].

Primary roots are the first root to emerge in both
dicots and monocots, and are derived from embryoni-
cally formed meristematic tissue. The root itself
broadly consists of xylem and phloem within a central
vascular column and pericycle to constitute the stele,
surrounded by concentric layers of epidermal, cortical
and endodermal tissues (figure 1c,d). Primary and
mature roots contain meristematic tissue at the tip
(the root apical meristem), which forms the basic
cell stem pool for other cell types in the root. Within
the root apical meristem, there is an area of rarely
dividing cells, the quiescent centre, which signals to
surrounding cells to organize and maintain the popula-
tion of initial stem cells [20].

In terms of the organization of the root tissue
itself, there are some substantial differences between
Arabidopsis and other species, including cereals. The
organization of root tissues for many other dicot species,
e.g. Brassica species, is generally not well characterized
at present. The majority of root architecture research in
monocots has focused on the species Zea mays and
Oryza sativa [29], thus these will henceforth be used
as models for monocot root systems for the purpose of
this review. Overall, cereal root tissues tend to be larger
and more complex; the primary root of cereals such as
maize or rice can have 10–15 cortical cell layers
(figure 1d) compared with the single layer typically
found in Arabidopsis (figure 1c), and often the root
quiescent centre population is drastically larger in size:
800–1200 cells in maize, compared with 4 in Arabidopsis
[30]. For a more detailed comparison, the reader is
directed to Hochholdinger & Zimmermann [30].

At the very tip of the root is the root cap, and colu-
mella cells, which are involved in root gravitropism—an
important growth aspect which causes roots to grow
deepner into the soil. Some columella cells, the statocytes,
contain specialized amyloplasts (organelles containing
dense starch). When a gravity stimulus is applied, sedi-
mentation of amyloplasts on a cell edge is thought to
result in a change in the distribution of auxin transpor-
ters in the cell membrane. This leads to changes in
auxin flux and a differential growth response within the
root, which in turn bends towards the gravity stimulus
[31]. In this issue, Guyomarc’h et al. [32] discuss the
importance and control of lateral root gravitropism.

Root branching is essential to increase the surface
area of the root system, enabling the plant to tap
more distant reserves of water and nutrients and
improve soil anchorage. In contrast to primary roots,
lateral roots are formed post-embryonically. In Arabidopsis and most dicots, the pericycle—a tissue layer located between the central vascular cylinder and the endodermis—represents the initiation site for lateral root branching (figure 1c). Specifically, the pericycle cells adjacent to the xylem pole represent the precursors to lateral root founder cells [33]. Lateral root initiation is the result of auxin-dependent cell cycle progression in a specific subset of the pericycle cells, to form ‘founder cells’. These initial cells subsequently go through several rounds of cell division to form the new lateral organ, which emerges perpendicularly to the parent primary root [34,35]. In contrast to the formation of shoot lateral organs, the later stages of lateral root emergence require the de novo creation of a new apical meristem within the emerging lateral root.

The reliance on the embryonically derived primary or ‘taproot’ system is a feature typical of dicots, such as Arabidopsis (figure 1a). Other dicots, for example Brassica, usually have a broadly similar root system in terms of development and morphological layout to Arabidopsis, though their comparatively longer life cycles usually result in a larger and denser root system, especially for perennial species. It should also be noted that considerable variety exists in root architecture between many dicot species, and Arabidopsis is (as in many areas of research) a simplified model.

Although cereals and other monocots form primary and lateral roots in a manner roughly similar to dicots, overall root architecture is more complex in monocots, forming a ‘fibrous’ root system of many types of branched root. In cereals, shoot-borne ‘crown’ and
‘brace’ roots, sometimes together with ‘seminal’ roots, constitute the majority of the monocot root system (figure 1b). These monocot-unique roots (particularly crown roots) are sometimes described as adventitious roots, but this is somewhat inaccurate as they are formed during the normal developmental programme of cereals [36]. The maize primary root can be seen from 2–3 days post germination, shortly followed by seminal roots by approximately 7 days after germination (figure 1b); similar to the primary root, these are formed embryonically, and emerge from the scutellar node [36]. Post-embryonically formed cereal roots include crown roots and brace roots. Crown roots start to emerge from below-ground stem nodes by 5–10 days post-germination. By contrast, brace roots emerge from above-ground stem nodes much later, approximately 6 weeks after germination [36,37]. Lateral root initiation also differs somewhat in cereals: both pericycle and endodermal cells can progress to become lateral root cells, and lateral roots form at the phloem poles rather than the xylem poles [38]. Furthermore, Arabidopsis has only two xylem poles (figure 1c), with lateral roots initiated in an alternating left–right pattern at each pole, but in cereals there can be as many as 10 or more phloem poles (figure 1d), resulting in a much more radial branching pattern around the parent root than observed in Arabidopsis [39]. In this issue, Jansen et al. [40] and Babé et al. [41] further explore lateral root initiation in cereal crops.

4. CONTROL OF ROOT BRANCHING IN ARABIDOPSIS

(a) Hormones

Lateral root initiation requires that primed pericycle founder cells undergo several rounds of cell division to form the lateral root primordium that gives rise to the new lateral root. The overriding influence in cell cycle regulation during root formation is the phytohormone auxin; many factors which influence lateral root branching can be observed to do so either directly via auxin, or by having a synergistic or antagonistic interaction with auxin (e.g. brassinosteroids and cytokinin). Indeed, simple exogenous application of auxin alone is sufficient to initiate lateral roots [42–44].

Lateral root initiation sites are created following the concentration of auxin in defined pericycle regions; the pericycle cells encompassed by these maxima are then ‘primed’ to undergo the divisions necessary for lateral root primordium formation [45]. Auxin promotes the interaction of members of the auxin/indole-3-acetic acid (Aux/IAA) family of repressor proteins with TIR1/AFB family auxin-receptor proteins. TIR1 is the F-box component of an ubiquitin ligase complex, which ubiquitinates Aux/IAAs marking them for destruction by the 26S proteasome. This frees the transcriptional regulators Auxin Response Factors (ARFs) from repression and allows them to bind to genes that contain auxin response elements, which are involved in controlling the organogenesis cell division programme [46–48]. Predictably, because the auxin-signalling pathway is so crucial for lateral root formation, several mutants for the various modules of this pathway display altered root phenotypes. For example, studies have isolated various mutants for the Aux/IAA and ARF modules of the auxin response pathway, such as the solitary root (slr) mutant, which has a complete absence of lateral roots owing to a gain-of-function mutation that increases the repressive activity of IAA14 [49]. In addition to slr, other gain-of-function auxin/IAA mutants also exhibit lateral root phenotypes: the short hypocotyl2-2 (shy2-2)/iaa3 and iaa28 Arabidopsis mutants both have reduced lateral root numbers [50,51]. Conversely, the gain-of-function auxin resistant2-1 (arx2-1)/iaa7 mutant demonstrates an increased number of lateral roots, suggesting that different members of the AUX/IAA family may have varying responses to auxin [52].

Simple initiation of cell division is, however, not enough; cell divisions must be tightly spatially and temporally controlled to give rise to correctly patterned primordia. Inducing pericycle cells to re-enter the cell cycle through overexpression of D-type cyclins or the G1–S transition-promoting transcription factor E2Fa/DPa is not sufficient to induce lateral root organogenesis in the absence of further cues determining cell patterning [53]. This control is achieved by fine-tuning the auxin response pathway through AUX/IAA–ARF pairs. Different combinations of these partially account for the diversity in phenotypic responses generated in response to a generic auxin signal [54,55]. SLR/IAA14 paired with ARF7–ARF19 is one such auxin response module, which directs transcription of the early auxin response lateral organ boundaries/asymmetric leaves-like (LBD/ASL) genes, which encode transcription factors for a set of genes controlling cell proliferation and patterning. In turn, these genes direct the initial asymmetric pericycle cell divisions during lateral root formation [56]. Another factor required for correct cell patterning downstream of the SLR/IAA14–ARF7–ARF19 auxin response module is the BODENLOS (BDL)/IAA12-MONOPTEROS (MP)/ARF5-dependent module, which has both lateral-root-promoting and -inhibitory mechanisms [57]. The ARF protein MP was recently demonstrated to control both its own expression and the expression of its repressor, the AUX/IAA protein BDL, subject to a threshold level of auxin, suggesting that the ARF–AUX/IAA auxin response may be self-regulating [58]. In this issue, Goh et al. [59] also highlight the roles of multiple AUX/IAA–ARF modules in lateral root development.

It has traditionally been thought that the pericycle cells require a de-differentiation stage with exit from an arrested G2 phase before renewal of cell division can occur [60,61], but observations suggest that the period between pericycle cell exit from the root apical meristem and the formation of founder is relatively short [62], thus allowing cells to remain division-competent without the requirement for de-differentiation. However, it does seem to be the case that a subset of pericycle cells remain in G2 phase for an extended period of time after exit from the meristem, and it is these cells which are most susceptible to lateral root initiation [64]. The founder pericycle cells can thus be considered to belong to an ‘extended meristem’ of not-yet fully differentiated pericycle cells. In this issue,
Parizot et al. [67] discuss this and highlight the heterogeneity of the pericycle.

The brassinosteroid regulatory pathway appears to act in synergy with the auxin pathway, with brassinosteroid-insensitive mutants exhibiting reduced lateral roots [68]. It is thought that brassinosteroids promote lateral root development through enhanced acropetal auxin transport, although the exact mechanism by which it does so is unclear.

Cytokinin is primarily synthesized in the root cap [69] and has a largely antagonistic effect to auxin in terms of root architecture: cytokinin has an inhibitory effect on lateral root branching, with mutants for cytokinin biosynthesis and sensitivity demonstrating increased numbers of lateral roots [70–73]. Auxin is synthesized primarily in young aerial tissues and undergoes polar transport towards the root tissues [74]. It is then distributed in the root tissues via strategic positioning of auxin carriers, particularly the auxin efflux carrier PIN-FORMED (PIN), which directs auxin flux enabling concentration in specific regions [75,76]. The inhibitory effect of cytokinin on lateral root branching appears to be due to cytokinin-mediated downregulation of PIN gene expression. PIN family auxin efflux facilitators are required to generate the targeted auxin maxima necessary for priming pericycle cells to form correctly patterned lateral root primordia [77]. In this issue, Bie lach et al. [78] identified various mutants that are impaired in the auxin–cytokinin crosstalk.

Increased synthesis or signalling of the gaseous phytohormone ethylene has an inhibitory effect on lateral root formation. Similar to cytokinin, this is thought to occur by altering auxin dynamics in the root, for example by modulating the action of the auxin influx carrier AUX1 [79,80]. Recently, treatment of Arabidopsis root tissues with the ethylene precursor 1-amino cyclopropane-1-carboxylic acid was also found to increase expression of the PIN3 and PIN7 auxin efflux transporters, thus promoting auxin transport towards the root apex and preventing the localized auxin accumulations needed to initiate lateral root formation [81].

Abscisic acid (ABA) is a phytohormone that has an important role in stress, particularly in stress responses to drought, as well as dormancy. In addition to closure of stomata to reduce water loss by transpiration, one ABA-mediated response to drought is the elongation of the primary root; more recently, evidence is growing that ABA also regulates lateral root branching, including in normal, non-drought conditions [82]. ABA signalling appears to connect many factors known to inhibit lateral root number and elongation to the resultant phenotype: for instance, lateral root inhibition in response to homogeneously high soil nitrate [83,84] and the inhibitory effect on lateral root development observed as a result of high (sugar) carbon:nitrogen ratios in the soil [85,86].

Strigolactones are a novel class of phytohormones, which are proposed to be synthesized from carotenoids in the root tissues [87,88]. Strigolactones are known to inhibit shoot branching, as well as being involved in interactions with root parasitic plants and symbiotic arbuscular mycorrhizal fungi [89]. However, it has recently become apparent that strigolactones are also involved in root morphogenesis: plants which are insensitive to strigolactones or deficient in strigolactone biosynthesis exhibit reduced primary root lengths with increased root branching, while application of a synthetic strigolactone analogue rescues the root phenotype of synthesis-deficient plants. The action of strigolactones on root phenotypes is thought to be due to modulation of auxin flux in the root, via strigolactone-regulated membrane cycling of PIN auxin efflux carrier proteins [90,91].

(b) Other signalling mechanisms

Various other root architecture-affecting mechanisms have been described, and we will highlight a few examples here. Although much emphasis has traditionally been placed on the roles of hormone signalling in root development, there are also many non-hormonal signalling pathways involved, such as peptide signalling [34,92,93].

The most prominent signalling system is through receptor-like kinases (RLKs). One of these is the RLK ARABIDOPSIS CRINKLY 4 (ACR4), thought to be a receptor for an as-yet undetermined small peptide signal. Following the first asymmetric pericycle cell division in lateral root formation, the small daughter cells formed can be observed to express ACR4; the expression of ACR4 continues in daughter cells following the second division, leading to a core-specific expression pattern [34]. In a cell autonomous fashion, ACR4 appears to play a role in promoting the early formative pericycle cell divisions required to initiate lateral roots; conversely, in a non-cell-autonomous fashion, ACR4 restricts supernumerary cell divisions in pericycle and columella cells once organogenesis has begun. This dual function, therefore, forms the basis of a homeostatic mechanism to prevent the unregulated division of pluripotent cells. More details on the role of peptide–receptor kinase signalling in root development will be highlighted further on in this issue by Stahl & Simon [94].

There are many other factors driving and controlling lateral root development, such as ABERRANT LAT- ERAL ROOT FORMATION 4 (ALF4) and ARABIDILLO-1 and -2. Arabidopsis ALF4 is a nuclear-localized protein, the expression and subcellular localization of which is independent from auxin influence. ALF4 appears to function by keeping xylem pole pericycle cells in a mitotically competent stage, possibly by preventing terminal differentiation of these cells [95]. ARABIDILLO-1 and 2 are Arabidopsis proteins with homology to the animal Armadillo/beta-catenin proteins: arabidillo–1/2 mutants have fewer lateral roots than wild-type, whereas ARABIDILLO overexpressing lines produce more. How ARABIDILLO precisely regulates lateral root branching is unclear, but it is possibly achieved through targeting of a lateral root inhibitor for ubiquitin-mediated destruction via the F-box of ARABIDILLO. This suggests a signalling pathway in lateral root initiation entirely separate from other known pathways [96].

(c) Impact of the environment on the root system

The environmental factors affecting root growth, such as nutrient and water availability and distribution

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(c) Impact of the environment on the root system

The environmental factors affecting root growth, such as nutrient and water availability and distribution
status, soil density, salinity and temperature, and microorganism interactions, are variable and complex; thus roots are necessarily plastic in their architecture. This plasticity is particularly well demonstrated in soil nutrient foraging. Nutrients such as nitrates (particularly NO₃⁻ but also NH₄⁺) and inorganic phosphates are distributed heterogeneously in the soil: indeed, root activity itself exacerbates soil nutrient heterogeneity. Plants, therefore, exploit concentrated nutrient reserves through modification of root architecture: for example, through the preferential elongation of lateral roots in both locally concentrated nitrate, and even more markedly, in phosphate-rich patches, respectively [65,97].

Nitrogen, sourced from the plant-available form of soil nitrates, is required by crops for the formation of essential macromolecules, including DNA and proteins. Many studies have demonstrated a relationship between soil nitrate levels and root architecture: lateral root growth is inhibited at low concentrations of soil nitrate, whereas locally nitrate-rich soil patches stimulate elongation of lateral roots in order to increase N uptake [98]. However, it is important to disentangle nutritional effects from signalling effects when considering soil NO₃⁻ in root phenotypes.

Recently, a mechanism to explain the inhibitory effect of low soil NO₃⁻ concentration on lateral root elongation has been described, via the NRT1.1 nitrate transporter [99]. NRT1.1 has a dual function: it is a sensor for soil NO₃⁻ concentration, and perhaps surprisingly, it also facilitates auxin uptake, thus coupling nutrient sensing with a hormonal response [100–102]. At low NO₃⁻ concentrations, NRT1.1 modifies auxin transport, promoting basipetal transport away from the lateral roots and thus inhibiting their formation and growth (although low nitrate concentrations can also inhibit growth simply owing to N starvation, independently from the signal-based effect of NO₃⁻ on NRT1.1). Furthermore, high NO₃⁻ levels also inhibit the auxin transport activity of NRT1.1, explaining the inhibition of lateral root formation and growth also observed when a high concentration of soil NO₃⁻ is distributed homogenously in the soil. Conversely, primary root growth in Arabidopsis appears largely insensitive to soil NO₃⁻ concentration [97], though increased primary root elongation in maize roots exposed to moderately high nitrate levels has also been reported [103].

Phosphorus is an important micronutrient that is often limiting because of the tendency of phosphorus to form insoluble compounds in the soil. In contrast to nitrate, which is highly mobile in soil and tends to leach easily, phosphorus also has poor soil mobility and tends to be concentrated in the upper soil strata [13]. Plants grown in low phosphorus conditions typically exhibit reduced elongation of the primary root and increased lateral root density and length [104]. This is thought to be an adaptive response to aid nutrient foraging. Indeed, another type of root, ‘cluster roots’, comprising a morphology of dense clusters of rootlets from a parent root, can be observed in some species, most notably members of the Proteaceae family. These cluster roots are formed as part of the normal root developmental programme rather than as a responsive change [105], and are an example of a root structure which appears to have evolved primarily for the maximization of phosphorus acquisition from the soil [106,107]. In this issue, Nussaume et al. [108] discuss a novel imaging technique for the visualization of nutrient uptake.

Drought has a major effect on root architecture, with many plants preferentially increasing primary root elongation and suppressing lateral root branching in response to drought. Many plants adapted to drought, such as sorghum, have a naturally more vertically orientated root structure [109]. Soil salinity also has an effect on root architecture owing to the infliction of osmotic and ionic stress on the roots, but in contrast to drought stress, high soil salinity inhibits primary root elongation while promoting lateral root emergence in glycophytes such as Arabidopsis [110,111]. Drought is often associated with high temperatures; in this issue, Hund et al. [112] discuss the effects of high soil temperature on root growth in maize.

The effects of soil pH and soil strength are less well understood. High soil acidity can solubilize and thus increase the availability of aluminium in the soil, to a level where aluminium becomes toxic to plants; studies in grasses and cereals have reported generally reduced root elongation in response to aluminium toxicity owing to acid soil [113,114]. Similarly, hard and compacted soils generally also impede root growth, particularly lateral root growth [113,115,116]. Soil strength is a complex factor in root growth: as well as increasing resistance and thus decreasing root growth, other stresses such as water stress, nutrient stress and root hypoxia may be induced as the soil is increasingly compacted [115]. The structure of soil is also important: an absence of pores in compacted soil can present problems, as roots typically use pores in the soil to facilitate growth, anchorage and root hair expansion [117].

**5. MOLECULAR CONTROL OF ROOT BRANCHING IN (CEREAL) CROPS**

While a lot of progress has been made on the level of the molecular control mechanisms of RSA, and specifically root branching, in Arabidopsis, comparatively less is known in cereals. The majority of research conducted in cereals has focused on the species O. sativa and Z. mays.

The rootless concerning crown and seminal roots (rcs) maize mutants, and their rice homologues, adventitious rootless 1 (arl1) and crown rootless 1 (crl1), completely lack all shoot-borne roots and also seminal roots in maize, leading to a reliance on the primary and lateral root system more reminiscent of that found in dicots. In the rice mutants, emergence of lateral roots from the primary root is also greatly reduced. Without support from shoot-borne roots, lodging (flattening) of the mutant plants is a major issue. Both maize rcs and rice arl1/crl1 have been found to be closely related to the Arabidopsis genes LBD16 and LBD29, two lateral organ boundary genes activated by ARF7/ARF19 [56,118,119]. In this issue, Majer et al. [120] describe the control of RTCS on ARF34 expression in maize.

Lateral rootless 1 (lrl1), rootless with undetectable meristems 1 (run1), short lateral roots 1 (srl1) and srl2 are
four maize mutants demonstrating abnormal lateral root development. The \textit{brt1} [121] and \textit{run1} [122] mutants fail to initiate lateral roots (in \textit{run1} at least this appears to be due to disrupted auxin transport) whereas \textit{slr1} and \textit{slr2} [123] are affected after initiation, leading to reduced lateral root elongation.

Mutants for crown root elongation have also been found in rice: the \textit{crl5} mutant for an AP2/ERF family transcription factor and as such is unable to induce the cytokinin type-A response regulator OsRR1, which usually negatively represses cytokinin signalling. In the absence of this repression, cytokinin inhibits crown root initiation in rice—similar to the inhibition of lateral roots by cytokinin observed in \textit{Arabidopsis} [124]. OsGNOM is a guanine nucleotide exchange factor for a GTPase—ADP-ribosylation factor (ARF)—involved in polar auxin transport, and has a high degree of homology to the GNOM protein found in \textit{Arabidopsis}. Mutants for OsGNOM also have defects in crown root development owing to disrupted distribution of auxin in the shoot tissues at the site of crown root primordia initiation [125].

\textit{Rtcs}, \textit{ar1l/crl1} and Osgnom are selected examples of the translatability of \textit{Arabidopsis} research into crop plants, but the added complexity of the monocot root system means the translation is not straightforward. The inadequacy of \textit{Arabidopsis} as a model organism in the context of studying shoot-borne root developmental processes is clear: all mutants discovered thus far for lateral root phenotypes in monocots are affected only in the embryonic roots [126,127]. This demonstrates that the mechanisms controlling architecture must be different for the post-embryonic and embryonic roots, respectively. However, despite some knowledge of shoot-borne root developmental processes in cereals such as maize and rice, there are currently no known mutants that demonstrate aberrant lateral root formation from shoot-borne roots in cereals.

One possible candidate model that may help bridge the gaps between \textit{Arabidopsis} and current cereal models is \textit{Brachypodium distachyon}. This species has many of the same benefits of \textit{Arabidopsis} as a model species, such as small size, relatively short life cycle and small genome size; but crucially it has the root architecture typical of monocots, because it belongs to the family Poaceae, which also includes rice, maize, wheat and barley. Pacheco-Villalobos \textit{et al.} [128] and Ingram \textit{et al.} [129] further discuss the suitability of \textit{Brachypodium} as a model for root studies.

### 6. VISUALIZING COMPLEX ROOT SYSTEM ARCHITECTURE

The study of root architecture has always been compromised by the inherent difficulties in studying a system that necessarily operates in a below-ground environment. Extracting an entire root system from soil, while maintaining completeness and avoiding damage to the finer elements of the root system, is a challenge (though some results have been generated this way, for example via ‘shovelomics’, a method of excavating, washing and phenotyping crown roots [13,130]).

For this reason, several other root growth methods have been trialled, such as growth on moistened germination paper rolls or pouches [131,132], sand rhizotrons [133] and gel-based systems [134–136], where phenotypic effects can be imaged using flatbed scanners, digital cameras or even lasers [137]. In this issue, Wells \textit{et al.} [138] present novel image acquisition and analysis methods to capture root growth and development. However, these methods are still not an adequate solution, because root architecture is influenced by many soil properties, including mechanical strength and density of the soil, the presence of air pockets in the soil, soil pH and temperature, and a variety of nutrient and biotic-interaction factors, which cannot be easily reproduced in highly artificial growth systems. The challenge of studying roots in the soil therefore remains, because it may be the only way to generate truly meaningful observations.

One strategy has been the use of X-ray computed tomography (CT) [139–141] which has the capability to visualize root architecture in situ by collecting many cross-sectional image ‘slices’ from which a complete three-dimensional root system image can be reconstructed, with no damage to the root system. This technique can be used to study root architecture under many different nutrient, moisture and soil density conditions in a physiologically relevant way. Less extensively, nuclear magnetic resonance (NMR) has been used in a similar way to CT to study root architecture in situ; however, CT is the preferred method owing to reservations about paramagnetic interference from ions in iron-rich soils [139]. In both CT and NMR systems, cost is a major drawback.

An alternative approach is mathematical simulation and modelling. From the starting point of programmed ‘rules’ based on direct phenotypic observations and molecular data, mathematical modelling can prove useful for mapping and integrating root growth and interactions in response to a variety of variables, and has been used to predict root growth patterns under differing conditions, including response to water and nutrient supply [142], various phosphorus concentrations [137], root adaptation to low nitrogen soil under carbon flux modifications [143] and the formation of root cortical aerenchyma in response to soil nutrient status [144], to name just a few. Simulation packages specifically for the study of root architecture have been developed, such as \textit{SmiRoot} [145]. Modelling approaches have been criticized in the past for being over-simplistic and failing to take into account the many complex variables of the soil environment and hydraulics [146]; nevertheless, recent progress in elucidating the biological, chemical and physical processes affecting root growth in soil means that newer models are now better equipped to integrate complex arrays of variables into one mathematical framework than was previously possible [29,147].

### 7. CONCLUSION

While the past decades have resulted in extensive insight into RSA, mainly in the model plant \textit{Arabidopsis} but also in some (cereal) crops, there are still various aspects of root growth and branching that need to be addressed.
This issue will provide answers to some outstanding questions, offer new tools to tackle remaining challenges, but also light the way for further studies.

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