# The role of plant root systems in evolutionary adaptation

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## Abstract

Roots provide a means to plants for gathering belowground resources. They are plastic and can adapt to ever-changing environmental cues. The plasticity of the roots comes from their ability to branch out by developing lateral and/or adventitious roots. In this chapter, we make an attempt to document the diversity in plant root systems and understand their role in evolutionary adaptation. After a brief introduction to different root systems, such as homorhizic and allorhizic ones, the relationship of plant roots with their surroundings, i.e. the rhizosphere and its effect on adaptation, will be discussed. Despite the difficulty to conclusively construct a timeline of evolution of plant root systems, documented facts from previous publications are examined and an effort has been made to delve into how rooting structures in plants adapted to prevailing conditions by bringing about endogenous changes vis-à-vis evolutionary development and exogenous changes to their surroundings.

## Introduction

Plant evolution is usually characterized as descriptive biology with the aim to explain how the present species diversity arose over a geological time frame. Studying morphological traits is a prime feature of paleobiology and still leads to fundamental insights (Hetherington et al., 2016a). However, paleontologists are highly dependent on the fossil record which is often very scarce, particularly for root specimens, that preserve very poorly (Raven & Edwards, 2001). With the advent of next generation sequencing techniques and the massive drop in sequencing costs, it becomes increasingly feasible to answer questions that bear relevance to evolutionary biology. For example, due to an increase in the number of fully sequenced plant genomes, it is possible to refine the phylogenetic tree, as recently been exemplified by Puttick and colleagues (2018). Their analysis of bryophyte transcriptomes suggested monophyly for the bryophytes (hornworts, liverworts, and mosses, Figure 2), a hitherto unanticipated finding (Rensing, 2018). Comparative analysis on a genome scale will also enable researchers to discover whole-genome duplications, events known to be major drivers of plant speciation (Li et al., 2015). Moreover, population-scale approaches such as genome-wide association studies (GWAS) offer the possibility to connect quantitative traits (plant height, yield or resistance) with the underlying molecular framework. Engineering plant root systems provides a means to react to rapidly altered environmental conditions and changes in soil characteristics of different habitats (Li et al., 2017; Uga et al., 2013). In this review, evolution of plant root systems is in the spotlight as an example of the above-mentioned advances in technology. In the first part, the plant root and root systems will be introduced to set the stage for the following complex details needed to appreciate the «news» from the fossil record. Subsequently, we look at root systems across the phylogenetic tree and provide the latest insights into their root system architecture (RSA) and adaptive capacity.

## Roots, root systems, and root biotic colonization

## What is a root?

Roots are one of the earliest adaptations that enabled plants to colonize land (Kenrick & Crane, 1997). They perform essential functions necessary for the survival and development of plants, such as anchoring them to a substrate, providing mechanical support, storing photoassimilates, and absorbing water and nutrients (Fitter, 2002; Olsen & Kemper, 1968; Bellini et al., 2014).

In seed plants, the development of the primary root (PR) begins with the elongation of the radicle from the embryo during germination. The root apical meristem (RAM) is already defined during embryogenesis (Dolan et al., 1993; Scheres et al., 1994; Van Norman et al., 2013) and comprises a quiescent center (QC) surrounded by stem cells (initials) for the various root tissue types (Figure 1A).

The initials in the proximal meristem divide to form vascular, ground, and epidermal tissues, which undergo cell elongation and thus lead to the growth of the radially symmetric root. Despite its simplicity, the root of the model plant *Arabidopsis thaliana* is comprised of all canonical tissue types: overlying the central stele (xylem and phloem) are single cell layers each of pericycle, endodermis, cortex, and epidermis (Figure 1A; Grierson et al., 2014; Scheres et al., 2002; Van Norman et al., 2013). Certain epidermal cells produce root hairs - unicellular enations that increase the surface area for absorption of nutrients and water.

The distal meristem gives rise to the columella/root cap cells (Scheres et al., 1994), which not only lubricate and protect the root, but also perceive chemical cues (Newcombe & Rhodes, 1904; Raven & Edwards, 2001; Ferrieri et al., 2017; Huang et al., 2018; Kutschera-Mitter et al., 1998). A similar structure is not present at the shoot apical meristem. The embryonic primary root grows vertically downwards along the gravity vector, displaying strong gravitropism (Darwin, 1880; Su et al., 2017). Therefore, the plasticity of the root system is mostly dependent on the post-embryonically formed lateral (root-borne) and adventitious

(shoot-borne) roots (Malamy & Benfey, 1997; Bellini et al., 2014). Lateral root (LR) and adventitious root (AR) programs are similar but different, and thus a closer look is needed to distinguish them.

<Insert figure 1>

**Figure 1. Root and LR tissue organization leading to different root system architectures**. A) Schematic representation of the *A. thaliana* root from the root apical meristem (RAM), via the basal meristem and elongation zone to the differentiation zone, which harbours LR primordia and root hairs. B) Progression of LR founder cells through the eight stages of LR development; transition of the endodermis occurs between stages IV and V; cortex and epidermis remain turgid as seen in VIII top view, C-E) types of root system architectures: C) Fibrous root system of a rice plant. D) Soy bean seedling with a tap root. E) *Physcomitrella patens* with single-celled rhizoids. Images sources: Reused and modified with permission from A - Peret, (2017), B - Stoeckle et al., (2018), C - Sparks, (2017a), D - Tamang, (2018) and E - Sparks, (2017b)

## Lateral and adventitious roots

Since most insights into LR and AR development are gained from *A. thaliana*, we focus our description of the processes to this example. This dicot provides an excellent system to study roots due to a simple and predictable arrangement of cells in radial layers (Dolan et al., 1993).

LR formation starts at the basal meristem (Figure 1A) with an auxin-dependent pre-initiation event called ‘priming’, which grants xylem pole pericycle (XPP) cells an LR founder cell identity (De Smet et al., 2007). Upon activation by the plant hormone auxin, the LR founder cell starts differentiating by increasing its cell volume; concomitant migration of the nucleus precedes an asymmetric anticlinal cell division (Casimiro et al., 2001; De Rybel et al., 2010;

Dubrovsky et al., 2001; Vermeer et al., 2014). This marks the initiation of LRs and takes place in the elongation zone (Bellini et al., 2014; Casero et al., 1995). The origin of ARs is not as clear and differs across species. Both programs rely on the action of the auxin regulatory gene *SOLITARY ROOT* (*SLR*; *AUX/IAA*), as the gain-of-function mutant *slr* lacks both secondary root types. In contrast, *monopteros* mutants (lacking a functional *AUXIN RESPONSE FACTOR5*/*ARF5* gene) cannot make LRs but are able to go through the AR program. The few molecular studies available for AR formation suggest that distinct players [(*ARF17*, *ARGONAUT1* (AGO1)] act in the AR program, since the LR program of *arf17* or *ago1* mutants is not hampered (Bellini et al., 2014; Gutierrez et al., 2009; Przemeck et al., 1996; Sorin et al., 2005).

After initiation, the differentiating cells, now forming the LR primordium (LRP), undergo several rounds of anticlinal and periclinal divisions in a stereotypical pattern to form a dome-shaped primordium. According to Malamy and Benfey (1997), the LR process can be divided into eight stages (Figure 1B), with stages 1-4 taking place before growth through the endodermis and stages 5-7 when the LRP breaches the cortex and epidermis (Malamy & Benfey, 1997; Stoeckle et al., 2018). Further proliferation and beginning cell elongation gives rise to a stage 8, fully-emerged LR (von Wangenheim et al., 2016).

Auxin plays a major role in this process and in addition to LR priming and initiation, it is also involved in signaling the endodermal cells overlying the LRP to accommodate the growing LRP through controlled volume loss (Vermeer et al., 2014). Degradation of cell adhesions in tissues overlying the endodermis is also mediated by auxin, thus allowing the growing LRP to traverse cortex and epidermis (Swarup et al., 2008). LR research in other species is less advanced. It seems that many regulators are conserved and perform similar functions, but also differences have been observed. In maize and rice, for example, the endodermis takes part in

LRP formation and constitutes the new epidermal layer (Bellini et al., 2014). It also needs to be tested whether the mechanisms identified in *A. thaliana* also hold true in plant species that have a larger root system with more cell layers that need to be traversed.

Adventitious rooting lacks the deep understanding at the molecular level that we have for LRs. This is mostly because *A. thaliana* is producing ARs only under adverse conditions (etiolated hypocotyls, de-rooted seedlings) and research in non-model species lags behind (Steffens & Rasmussen, 2016). However, shoot-borne rooting is a very important horticultural and agronomical trait, and thus there is a body of knowledge about the impact of different treatments on their potential to induce ARs from a variety of species and tissues [*Fragaria* spp. (strawberry), *Malus pumila* (apple), *Vitis vinifera* (grapevine), *Humulus lupulus* (hops); stolon, leave, stem] (Verstraeten et al., 2014). The unifying theme is that there exists a huge variability in treatments that promote rooting in one species do not do so in others. Significantly, many hormones influence AR and LR in opposite directions. For example, the volatile plant hormone ethylene has been shown to promote AR formation, whereas it inhibits LR formation (Filip et al., 2007; Lewis et al., 2011; Muday et al., 2012; Negi et al., 2008, 2010; Ruzicka et al., 2007; Verstraeten et al., 2014). Most hormone application acts through modulating auxin fluxes and concentrations, which also acts as a key regulator in AR formation. Regardless, emergence of ARs seems to be inhibited rather than promoted by exogenous auxin application (Bellini et al., 2014).

As exemplified in *Oryza sativa* (rice) and *Solanum lycopersicum* (tomato), the formation of ARs is a way to react to environmental changes like flooding (Mcnamara & Mitchell, 1991). Long periods of hypoxia to the root system are detrimental, and thus being able to produce new roots from stems above the water level is a vital trait and contributes to the plasticity and flexibility of the RSA.

## Tap roots, fibrous roots, and root system architecture

Some of the most astonishing root systems that can be observed above ground are AR-based, such as mangrove stilt roots or aerial roots of *Hedera helix* (ivy) and epiphytic orchids. Less well known is that adult root systems of monocots (monocotyledons – embryo with one cotyledon), such as *Zea mays* (maize) and rice, only use ARs. Even in dicots (eudicotyledons – embryo with two cotyledon), such as tomato, *Vigna radiata* (mung bean) and *Helianthus annuus* (sun flower), ARs substantially add to the RSA.

RSA is defined by the amount, shape and organization of PR, LRs, and ARs (Smith & De Smet, 2012). In eudicots, the primary root tends to dominate, giving rise to LRs and forming an allorhizic root system, commonly known as the tap root system. Monocots have an ephemeral primary root, which allows other types of roots, such as seminal and adventitious roots derived from shoots, to dominate the root system. This is a homorhizic root system, commonly known as the fibrous root system (Seago & Fernando, 2013; Bellini et al., 2014).

During their life cycle, plants have to survive in the soil environment with a nutrient availability that is mostly heterogenous in space and time. An important role in the adaptive responses of plants to their ever-changing environment is thus their ability to shape the root system in order to efficiently extract nutrients and water from the soil. It is well known that excess or deficiency of different nutrients induces changes in RSA and root morphology, which can to some extent be attributed to specific nutrients (Giehl & von Wiren, 2014).

Mineral elements have complex interactions with themselves and other soil constituents, and can be carried out of the rooted soil volume by water. For a plant to be productive and have competitive success, it is important to be able to efficiently acquire these elements. In addition to nutrient status, very strong gradients in temperature, oxygen, water availability, pH, and bulk density occur with soil depth over a scale of centimeters (Lynch, 1995).

In order to get additional insights into how plants can modify their RSA to nutrient availability, the use of functional-structural plant models, such as *SimRoot*, can be very powerful. Using this approach, the 3-dimensional development of a maize RSA was simulated to predict the optimal root branching density for phosphorus or nitrogen uptake. This revealed that a high branching density with short LRs was optimal for phosphorus acquisition and sparse branching with long LRs was optimal for nitrogen acquisition. This experiment showed that there is no such thing as an optimal branching density because it depends heavily on the nitrate-to-phosphorus ratio in the soil environment. This means there is a RSA tradeoff in most soils: plants may have different optimal branching behavior in different soil domains (Postma et al., 2014).

In recent years, several studies have explored how the root system has adapted to local growth conditions. Most of these studies have been performed using *A. thaliana*, where the advent of cheap, large-scale, high-resolution genotyping and sequencing technologies has made the sequencing of many plant strains feasible (Alonso-Blanco et al., 2016). This has enabled GWASs to identify alleles responsible for a large variety of quantitative traits (Weigel, 2012) by measuring them in many different accessions and subsequently associating them with single nucleotide polymorphisms (SNPs). As these accessions have been collected from different geographical locations, the observed changes in RSA and general growth reveals how these traits might have played a role in the adaptation to their growth environment. GWASs have been successfully used to identify regulators of root growth under normal, different nutrient and hormone treatments, and stress conditions (Ogura & Busch, 2015; Julkowska et al., 2017; Meijon et al., 2014; Ristova et al., 2016; Rosas et al., 2013; Slovak et al., 2014). In addition, it was reported that natural *brevis radix* (*brx*) loss-of-function alleles confer adaptation to acidic soils in *A. thaliana* (Gujas et al., 2012). Although screening of different accessions has been mostly used in *A. thaliana*, this approach was shown to also have a big potential for

understanding the role of RSA in the adaptation of rice to drought (Uga et al., 2013). With the rapidly evolving sequencing technologies, we expect that GWAS with more plant species (including crops), and comparisons between wild and domesticated species, will yield many new insights into how the evolution of RSA has contributed to adaptation. As most of the studies have relied on plate-based analyses of growth and RSA phenotypes, it is clear that we need to grow and phenotype roots of plants growing under near physiological solutions in order to gain better insights into how RSA can be exploited for successful adaptation to different environmental conditions. Recent advancements in X-ray micro-computed tomography and bioluminescence-based systems like the Growth and Luminescence Observatory of roots (GLO-roots) will be important to get a more complete picture of how dynamic RSA plays a role in the adaptive responses of plants (Mairhofer et al., 2015; Rellan-Alvarez et al., 2015).

## Rhizomes and rhizoids

Flowering plants like hops, *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), and *Iris* spp., as well as non-flowering plants like horsetails, ferns and lycophytes, have a belowground structure called rhizome, which is commonly confused to be a part of the root system. Although rhizomes are anatomically not a root, they do fulfill root functions. In order to increase the surface area, rhizomes mostly bear single-celled rhizoids, a structure that anatomically and molecularly closely resembles root hairs (Jones & Dolan, 2012). However, different in origin and shape, rhizomes and true roots are all subterranean and as such intimately connected and influenced by the rhizosphere with its microorganisms. Their influence on root systems is a major driving force of plant evolution with large geochemical implications (Pieterse et al., 2016; Schwartz et al., 2013).

## Mycorrhizae and Rhizobia

Plants have been accompanied by fungi, bacteria, viruses, and protista over millions of years, and these interactions are thought to have accelerated the adaptation to land (Bonfante & Genre, 2010).

Endophytic soil bacteria enter the plant root, colonize them and provide beneficial functions, such as promotion of plant growth, health, and systemic resistance (Ryan et al., 2008; Shahzad et al., 2010). They often secrete growth hormones like auxin, cytokinin and gibberellic acid, or reduce ethylene production and thus connect plant growth promotion with changes in RSA (Lugtenberg & Kamilova, 2013; Ortíz-Castro et al., 2009; Schwartz et al., 2013). The interaction between *Rhizobium* spp. and legumes is a prominent and the oldest known example of a symbiosis between bacteria and plants (Hellriegel & Wilfarth, 1888; Lavin et al., 2005). In exchange for carbon, these nitrogen-fixing bacteria provide plant-accessible ammonium made from atmospheric $N_2$. This process happens in so-called nodules. Nitrogen-fixing symbioses can be found amongst the Rosid 1 clade (incl. legumes; Soltis et al., 2000). Many agriculturally relevant plants, such as *Glycine max* (soy bean), *Cicer arietinum* (chickpea), *Pisum sativum* (pea), and *Trifolium* spp. (clover), interact with *Rhizobia* spp. As they have maintained this symbiosis for an extended period of time, it must have provided an evolutionary advantage in the adaptation to their environment. We anticipate many novel insights into plant-bacteria interactions as the systematic exploration of the root microbiome by high-throughput sequencing techniques advances.

Symbiosis between plants and fungi evolved over 400 million years ago, with arbuscular mycorrhizal fungi (AMF) being the oldest and most abundant (Field et al., 2015). The success of these connections is evident by the estimated 90% of land plants that interact with AMF (Bonfante & Genre, 2010). To colonize the land, symbiosis was an essential means to improve nutrient uptake (phosphorus and nitrogen) in exchange for carbon (Pirozynski & Malloch, 1975; Bidartondo et al., 2011; Chiu et al., 2018; Kiers et al., 2011; Martin et al., 2017; Wang

et al., 2010). AMF can influence the RSA of several plant species through an increase or decrease of root branching, and they can even overcome LR mutant phenotypes (reviewed in Gutjahr & Parniske, 2013). During evolution, the *Brassica* lineage (incl. *A. thaliana*) lost the ability to interact with AMF (Bonfante & Genre, 2010). However, recent studies revealed that the fungus *Colletotrichum tofieldiae* can support the phosphorus uptake of *A. thaliana* roots under phosphorus starvation conditions (Hiruma et al., 2016).

## From first roots to angiosperm root diversity

### Devonian rooting structures

Multicellular, non-vascular plants that can be described as being similar to extant bryophytes are accepted as the first colonizers of land close to lake shorelines during the mid to late Ordovician of the Paleozoic era (ca 450 Mya; Figure 2; Kenrick et al., 2012; Steemans et al., 2009), although a recent study has placed the event at a much earlier, middle Cambrian–Early Ordovician period (ca. 525 Mya; Morris et al., 2018). It is difficult to conclude what kind of roots these first land plants had because there are barely any plant body fossils for study from this period.

Initially, rooting structures developed to help anchor the plants, which moved from water to land. The first rooting structures were simple rhizoid-based rooting systems (RBRSs) that grew mostly horizontally on the surface and, when subterraneous, shallow on moist lowlands (Jones & Dolan, 2012; Algeo et al., 1995). The earliest fossil evidence of RBRS comes from Rhynie Chert in Scotland from the Devonian period (410 Mya; Dolan and Hetherington, 2017). Their role in evolutionary adaptation was to anchor the thallus to the substrate and assist in taking up nutrients (Kenrick & Strullu-Derrien, 2014). The liverwort *Marchantia polymorpha*, an extant bryophyte, is generally used as a model for the earliest terrestrial plants that gave rise to RBRSs

from rhizoid bearing axes of their gametophyte and sporophyte generation (Shimamura, 2016; Graham et al., 2004; Menand et al., 2007). *M. polymorpha* also exhibits two distinct rooting structures: tuberculate rhizoids scattered underneath the thallus of the gametophyte, mainly for anchoring the plant to its substrate, and smooth clustered rhizoids in the sporophyte, mainly for absorption of nutrients (Graham et al., 2004). Unlike *M. polymorpha*, the early land plants bore only one type of smooth rhizoids. They were filamentous and unbranched (Kenrick & Strullu-Derrien, 2014). When comparative studies were conducted on rhizoids of *M. polymorpha* and the moss *Physcomitrella patens* with root hairs of *A. thaliana*, it was found that a highly conserved molecular network controls the development of these cell types in all three species (Honkanen et al., 2017; Menand et al., 2007).

One of the commonly observed functions of roots in extant terrestrial plants is the ability to provide mechanical support. Shallow growing rhizoids could barely be expected to provide mechanical support. Instead, early vascular plants possessed subterranean rhizomatous axes which bore rhizoids (Kenrick & Strullu-Derrien, 2014). Study of the fossils of *Drephenophycus*, an early vascular plant, has revealed the importance of rhizomatous growth as an early precursor of deep-rooted plants. These belowground rhizomes were found to grow extensively and form complex subterranean networks. They could produce dense vegetation and protect the substrate surface from erosion (Xue et al., 2016). These rhizomes are similar in their clonal growth to some species of extant clubmosses and ferns of the genera *Lycopodium* and *Pteridium*, respectively, nicely tying in with the fact that some basal members of lycophyte and euphyllophyte clades still retain the feature of rhizomatous growth (Kenrick, 2013; Kenrick & Strullu-Derrien, 2014; Xue et al., 2016).

Owing to the lack of reliable fossils for a period of nearly 40 million years, the transition from RBRS to first true roots is poorly understood; hence, this is a highly debated and controversial

topic. Depending on the methods of investigation, few theories exist on the evolution of roots. It was proposed that roots evolved separately in different clades of early vascular plants (Kenrick & Strullu-Derrien, 2014). One theory suggests that roots evolved from rhizoid-bearing axes, while another suggests the possibility of leaves turning into roots (Bierhorst, 1971; Rothwell & Erwin, 1985; Fujinami et al., 2017). It has also been postulated that roots arose as *de novo* organs and not from any other plant part (Matsunaga & Tomescu, 2016). Fujinami and colleagues (2017) studied the diversity of RAMs in different species of extant lycophytes, which are believed to have diversified at the same time that roots evolved, reflecting the multiple origins of the root.

<Insert Figure 2 here>

**Figure 2. Key events and innovations that shaped plant evolution.** Land colonization (orange dot) happened at the transition from the Cambrian to Ordovician period. Vascular tissues (cyan dot) date back to the Silurian. Evolution of roots (red dots) happened independently in lycophytes and the ancestor of all euphyllophytes, and is set in the Devonian period. More than 200 million years later, first seed plants (violet dot) occur in the Jurassic fossil record. During the Cretaceous period, flowers arise in the angiosperm lineage.



## Paraphyletic origin of true roots

As plants started to get bigger, the rooting system also got more complex. This could be seen in lycophytes, the first vascular plants. Contrary to vascular plants from higher divisions, lycophytes have leaves with a single vascular bundle called microphylls instead of leaves with a more complex network of vasculature called megaphylls (Doyle, 2012; Kenrick, 2013). Organisms in this order are the club-, fir- and spike mosses that are commonly confused with ferns. Species belonging to the extinct genus *Zosterophyllum* were one of the first known

vascular plants to bear rooting structures intermediate to rhizoids and true roots. Studies of fossils have revealed that these plants had a below-ground rhizome that bore roots in dense tufts (Hao et al., 2010). The differentiating factor between early roots in lycophytes and the rhizoids in extinct bryophytes is that this was the first instance where structures similar to aerial stems grew belowground in a distinct opposite orientation to the aerial stems. However, they were narrower and shorter without any microphylls or leaves and bifurcating irregularly, suggesting that they developed to have a specific rooting function (Gensel et al., 2001). The meristems frequently found on rhizomes and erect stems that gave rise to these early roots were dormant and could develop into either shoots or roots (Kenrick, 2013). Fossil records of the cottonwood canyon lycophyte, an early basal lycophyte, exhibit regular branching of this belowground stem (K-branching) and suggest that LRs developed from these branches (Matsunaga & Tomescu, 2016). However, these were not true roots. While advancing our knowledge, studies like this especially highlight the parts of early root evolution that are still unknown. Nevertheless, based on developmental differences, there is a consensus in the community that roots have paraphyletic origin, i.e. they evolved independently at least twice: once at the base of the lycophytes and once in the euphyllophyte clades (Figure 2; Kenrick & Crane, 1997; Kenrick & Strullu-Derrien, 2014; Raven & Edwards, 2001). Prominently, lycophyte roots branch apically by meristematic division, while euphyllophyte roots originate endogenously from cells of the pericycle or endodermis (Friedman et al., 2004).

Another theory proposes further independent evolution of roots in euphyllophytes based on their origin during embryogenesis: in eudicots, the root is formed very early from the hypophysis of the eight-cell stage embryo while in monocots, it forms from the ground meristem of the globular stage embryo (Bennett & Scheres, 2010).

## The oldest root meristem

A major determinant to distinguish and categorize root fossils is the structure of the RAM. Lycophyte and fern RAMs are very distinct from those of the later euphyllophyte clades as they only have a single active meristematic apical cell of an inverted-prism structure. By dividing asymmetrically in three directions, the apical cell forms the tissue that later acquires radial symmetry. Euphyllophytes have RAMs of the above-mentioned structure (Figure 1A), with angiosperms having rather small organizing centers (few initials) while many proliferating cells surround the QC of gymnosperms.

It is extremely challenging to find these small (<2mm) organs fossilized. However, a recent study has accomplished this (Hetherington et al., 2016b). In the Oxford natural history collection, they found two specimens in a carboniferous rock that dates back 320 million years and show a remarkable state of preservation. Moreover, they found one RAM to be fossilized in an actively growing state (no differentiated vascular tissue close to the QC). This allowed them to perform modern lineage tracing analysis and led to the classification of the RAM belonging to the gymnosperm clade. By employing the "Körper-Kappe" theory, developed by the Swiss botanist Otto Schüepp (Schüepp, 1917), they reasoned that *Radix carbonica* is to be treated as a separate species (Hetherington et al., 2016b).

## Angiosperm root system plasticity

Among all the vascular plants currently inhabiting the earth, angiosperms form the most diverse group with 416 families and nearly 300,000 species (Christenhusz & Byng, 2016). The name literally means encased seeds, which happens to be a characteristic feature of angiosperms, differentiating them from gymnosperms. It is hard to formulate a common characteristic root system for angiosperms as the species range from aquatic lilies to woody magnolias. Additionally, most of these species are hardly studied with respect to their root systems, the

exceptions being monocots and dicots. Most of the world's economically important plant species belong to these two divisions and are, hence, well studied.

**Monocots**

Many economically important species, including members of the *Orchidaceae* (orchids), *Poaceae* (true grasses), *Arecaceae* (palms), *Musaceae* (bananas, plantains), *Zingiberaceae* (ginger), *Liliaceae* (lilies, tulips), and *Amaryllidaceae* (onions, garlic, daffodils) are monocots. Monocots exhibit one of the two major types of root systems seen in angiosperms, the fibrous or homorhizic root system. In this type of root system, the embryonic root that arises from the seed is only important during germination and early stages of seedling development. Later, post-embryonic roots that emerge from the shoot, take over the rooting functions (Bellini et al., 2014).

Unlike LRs, which always arise from pericycle cells, post-embryonic roots in monocots arise from different tissues in the shoot, and hence from different cell types. However, they always develop from cells neighboring vascular tissues, such as hypocotyl pericycle cells, phloem/xylem parenchyma cells, interfascicular cambium cells, or cells close/adjacent to the vascular cylinder of the stem. All embryonic and post-embryonic roots in monocots form LRs in order to branch out (Bellini et al., 2014).

Orchids represent 50% of the monocots and are an interesting case study with respect to the role of RSA in evolutionary adaptation. Orchids can be epiphytic or terrestrial. Epiphytic orchids produce aerial roots that anchor the plant to trees and, with an adaptive spongy epidermis called velamen radicum, absorb nutrients from the humid air. In contrast to the terrestrial orchids, epiphytic orchids do not make LRs. It was recently reported that two genes, *AGAMOUS LIKE12 (AGL12)* and *ARABIDOPSIS NITRATE REGULATED1 (ANR1)* could have played a major role in the segregation of root phenotypes in epiphytic and terrestrial

orchids as they were either absent or showed reduced expression in epiphytes (Zhang et al., 2017). Studies in *A. thaliana* have shown that *AGL12* is involved in root cell differentiation and *ANR1* is a key regulator of LR development in response to external nitrate supply (Parenicova, 2003; Zhang & Forde, 1998).

Beside the orchids, cereals represent another large group of the monocots and belong to the *Poaceae.* Maize, rice, and *Triticum aestivum* (wheat), the top three staple crops by global annual production, are all cereals (Gennari, 2015). They have complex and widely distinct RSAs and can bear different types of roots: in addition to PRs, they can form seminal roots (SRs), crown roots (CR), and brace roots (BR) (Feldmann, 1994). The development of these roots even within one species is firmly dependent on external conditions and varies between subspecies. There are also differences in total root mass per plant, number of root branches per unit length, lateral spread, and number of SRs (Feldmann, 1994; Pagès & Kervella, 2018). Photosynthesis type also seems to have an effect on root branching and root biomass (Atkinson et al., 2016). Among all monocots, the root system of cereals is the best described and studied because of their obvious economic importance. However, this knowledge tells us little about evolutionary adaptation of RSA in monocots due to human intervention in breeding modern varieties of cereals. Maize, rice, and wheat have all been domesticated by humans for thousands of years and, until recently, little to no consideration was given to root phenotypes during selection. In fact, cereals have been directly selected for higher grain yield, which led to indirect selection of weaker and smaller roots (Gaudin et al., 2014; Schmidt et al., 2016). A comparative study of two cultivated varieties of maize, sweet and popping corn, and teosinte (*Z. mays* subsp. *parviglumis*), the ancestor of modern maize, has revealed that the shoot-to-root ratio was significantly higher while root thickness was significantly lower in cultivated varieties compared to teosinte (Szoboszlay et al., 2015). Another study investigated the effect of altered dosage of an allele of *Teosinte branched1 (Tb1)*, a major domestication gene in maize that is

known to repress shoot branching on roots. It was reported that decreased expression of *Tb1* had a significant impact on RSA, with increased branching via an increase in the number of CRs and LRs (Gaudin et al., 2014). RSA in domesticated cereals has also been significantly altered by agricultural practices in recent times due to the increased use of fertilizers and irrigation. A comparative study of 16 varieties of cultivated maize developed in different time periods over the last 100 years was conducted to understand the effect of nitrogen levels in soil on RSA. BRs and CRs grew less steep and LRs grew longer in newer compared to older varieties, suggesting adaptation to better nutrient foraging and reduced water foraging (York et al., 2015). A similar result was observed in a comparison of the upland rainfed rice variety Azucena and lowland irrigated rice variety IR64. Azucena was found to have deeply rooted PRs and CRs, while IR64 was shallow rooted but highly branched, suggesting the tradeoffs made by the cultivated varieties depend on the conditions they were selected in (Clark et al., 2011). Rebouillat and colleagues (2009) described a remarkable adaptation to survive in waterlogged conditions, where LRs in rice form aerenchyma cells in the outer cortical layers to enhance the diffusion of oxygen. Increased awareness of the effect of indirect breeding on root phenotypes is leading to more efforts to create new varieties of cereals by introgression of genes that have been lost or altered during domestication (Dixit et al., 2012; Gaudin et al., 2011; Meister et al., 2014).

Wheat has been cultivated for over 10,000 years. During its domestication some traits were lost that were in fact important for survival (Zohary, 2004). A recent study describes an increase of SR number in tetra- and hexaploid wheat, but also a reduced number of SRs in tetraploid *Triticum dicoccum* (wild emmer) (Golan et al., 2018). Furthermore, this study revealed that the reduced number of the SRs is due to suppressed primordia in the wild form, and that this lower number is necessary to recover from temporal water stress. In general, deeper rooting varieties have an advantage in periods of drought (Manschadi et al., 2013). Temperature changes, as

well as root pruning in combination with a carbohydrate-rich nutrition, result in a different behavior of LR formation (Bingham & Stevenson, 1993).

One possibility to assess the effects of domestication on RSA evolution is to contrast the above finding with the model grass *Brachypodium distachyon*, which also belongs to the *Poaceae* and thus is a close relative of wheat and barley (Kellogg, 2001; Vogel et al., 2006). *B. distachyon* is a wild species that was never used in breeding (Girin et al., 2014). At present, the research community has access to over 200 natural accessions and many of them have already been sequenced (Gordon et al., 2017). In recent years, researchers started to investigate the RSA of *B. distachyon* and its natural variations (Pacheco-Villalobos & Hardtke, 2012; Ingram et al., 2012; Pacheco-Villalobos et al., 2013). It was shown that the root system of different *B. distachyon* accessions display different responses to phosphorous and nitrogen starvation conditions (Ingram et al., 2012).

**Dicots**

Dicots form a paraphyletic group, with basal angiosperms diverging earlier than the monocots did (APG IV, 2016; Stuessy, 2010). As mentioned earlier, the simple morphology of the root of *A. thaliana*, the vast genetic tools available in this model, and its accessibility for microscopy has made this species very suitable to study root development. The *A. thaliana* root system has a taproot with several orders of LRs. In recent years, a very detailed picture has emerged about the molecular and genetic pathways underlying LR development in this species, which we will, however, not discuss this in this chapter. For more information regarding the underlying developmental mechanisms we refer the reader to some excellent recent reviews (Otvos & Benkova, 2017; Stoeckle et al., 2018; Vilches-Barro & Maizel, 2015). One of the challenges for the coming years is to scrutinize to which extent the molecular mechanisms identified in *A. thaliana* are conserved in other plant species. For instance, in the legume *Medicago truncatula*,

LRs also initiate from the xylem pole pericycle and, in general, follow a similar developmental program. However, the overlying endodermis and cortex of *M. truncatula* will reactivate the cell cycle and will become an integral part of the newly formed LR (Bensmihen, 2015; Herrbach et al., 2014). This is also observed in other plants, such as tomato (Ivanchenko et al., 2006) and several cereals (Orman-Ligeza et al., 2013), suggesting that the system of *A. thaliana* might not serve as a stereotypical LR model. Interestingly, *M. truncatula* can also engage in a symbiosis with bacterial *Rhizobia* spp., leading to the formation of a new lateral organ, the root nodule. It has been suggested that this process has exploited the LR developmental program (Bensmihen, 2015). Interestingly, non-legume actinorhizal plants and *Parasponia* spp. form a different type of nodule that also initiates from the pericycle and has a structure much more similar to LRs, as these nodules have a central vasculature instead of the peripheral vasculature observed in legume nodules (Pawlowski & Bisseling, 1996).

In order to address how RSA could have played a role in the adaptation to the growth environment, it is important to compare RSA of plants grown in different conditions and to identify the mechanisms that are casual for these changes. It has been reported that there is a striking natural variation between different *A. thaliana* accessions, and that it is very likely that these changes occurred during the adaptation to their growth environment (Horton et al., 2012). Indeed, there is also substantial variation between the RSA in these different accessions (Meijon et al., 2014; Rosas et al., 2013). Preliminary results also suggest that this is the case for *B. distachyon* (Pacheco-Villalobos & Hardtke, 2012).

## Geochemical consequences of root evolution

With the advent of roots in the Devonian period, plants have become increasingly successful to colonize land. Due to their dependence on rock-derived minerals like phosphorous, potassium, calcium, magnesium and iron, plants possess the mechanism to rapidly weather

rocks to release these minerals. This process was accelerated by the evolution of embryophytes and their new rooting structures (Raven & Edwards, 2001). When early vascular plants started to colonize land in the late Ordovician period, they caused a massive drawdown of atmospheric $CO_2$, which was rapidly forming stable compounds with the minerals that had been exposed to the atmosphere. This led to a temporary but substantial drop in atmospheric $CO_2$ levels, plunging the earth into an ice age and causing mass extinction of marine organisms (Algeo et al., 1995; Lenton et al., 2012). In the late Devonian, plants innovated arborescence (tree-like stature). Plants belonging to the *Archeopteris* genus dominated the earth during this period, and they grew up to 30 meters in height. Increase in aboveground stature meant that the belowground biomass needed to reciprocate: roots increased in thickness and grew deeper into the earth. New geoclimate models also hint at a striking contribution of arbuscularization of roots to accelerate the change in geochemical conditions on the early planet earth (Mills et al., 2018).

## Conclusion

In conclusion, we like to revisit a statement made by Hetherington and colleagues (2016a), which was also reiterated and emphasized by Tomescu (2016) due to its importance for all the current and future scientists who are trying to reach conclusions about plant evolution: “extant anatomical diversity represents only a subset of all the diversity that ever existed”. Sampling only living specimen will thus not help us to improve and complete the bigger picture. Therefore, we need to embrace the possibilities the fossil record might hold, and look forward to the exploration of treasures from yet unexplored natural historic collections.

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## Figures

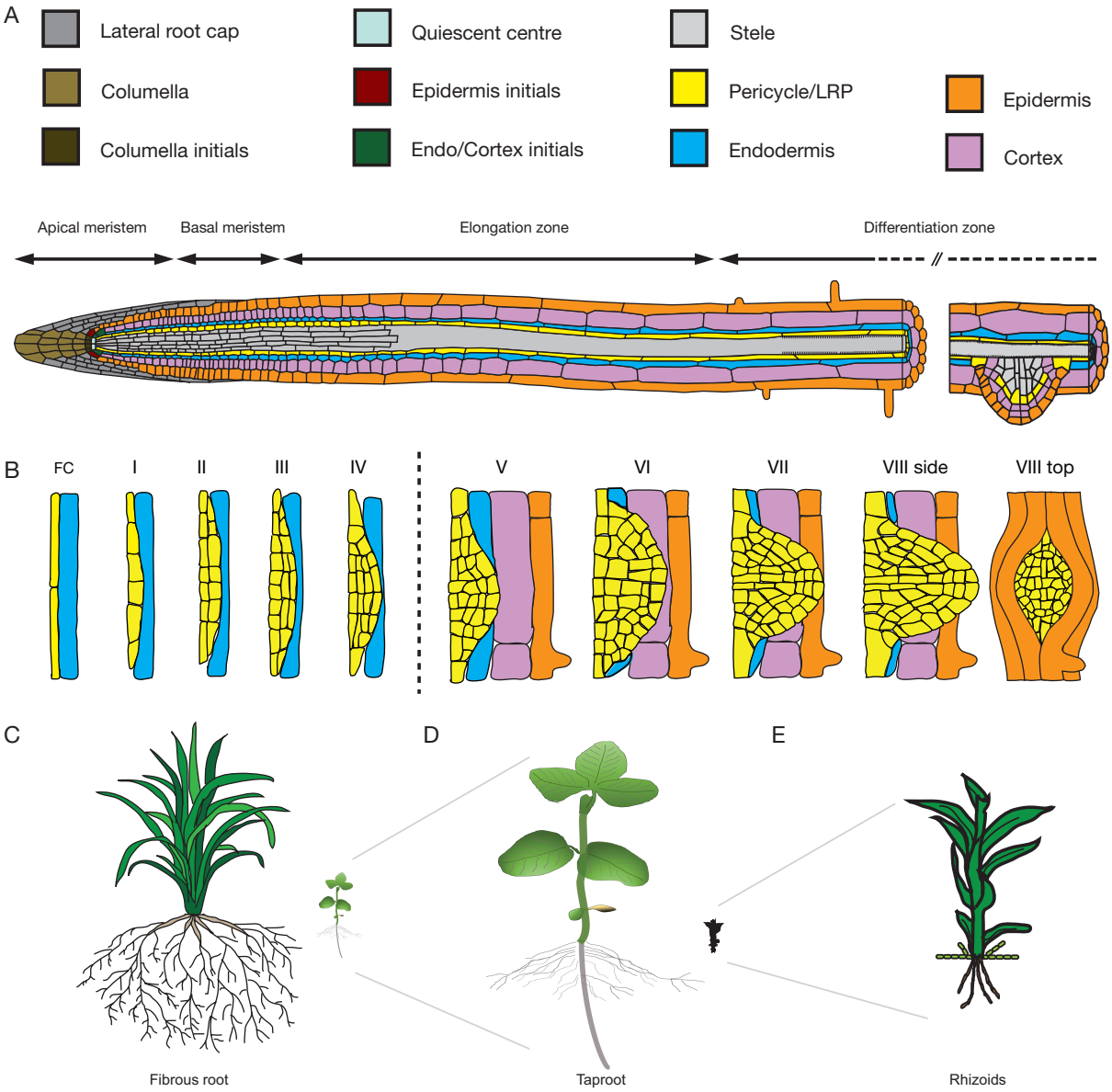


**Figure 1. Root and LR tissue organization leading to different root system architectures**. A) Schematic representation of the *A. thaliana* root from the root apical meristem (RAM), via the basal meristem and elongation zone to the differentiation zone, which harbours LR primordia and root hairs. B) Progression of LR founder cells through the eight stages of LR development; transition of the endodermis occurs between stages IV and V; cortex and epidermis remain turgid as seen in VIII top view, C-E) types of root system architectures: C) Fibrous root system of a rice plant. D) Soy bean seedling with a tap root. E) *Physcomitrella patens* with single-celled rhizoids. Images sources: Reused and modified with permission from A - Peret, (2017), B - Stoeckle et al., (2018), C - Sparks, (2017a), D - Tamang, (2018) and E - Sparks, (2017b)

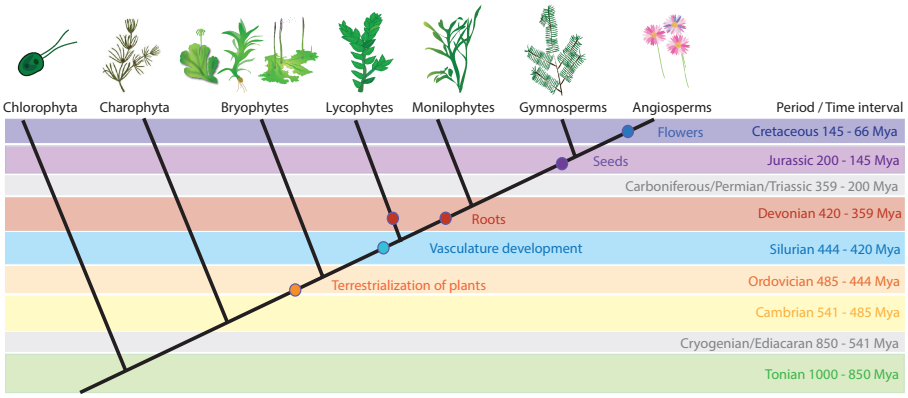


**Figure 2. Key events and innovations that shaped plant evolution.** Land colonization (orange dot) happened at the transition from the Cambrian to Ordovician period. Vascular tissues (cyan dot) date back to the Silurian. Evolution of roots (red dots) happened independently in lycophytes and the ancestor of all euphyllophytes, and is set in the Devonian period. More than 200 million years later, first seed plants (violet dot) occur in the Jurassic fossil record. During the Cretaceous period, flowers arise in the angiosperm lineage.