"Phenomics of root system architecture: Measuring and analyzing root phenes"

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Phenomics of Root System Architecture: Measuring and Analyzing Root Phenotypes

Plants cannot simply walk to more favorable environments. Behind this statement hides a complex and fascinating truth. Despite their immobility, plants still need to access soil resources such as water and nutrients to survive in environments that are continually changing. To do so, plants have evolved extensive and highly plastic root systems to forage in the surrounding heterogeneous soil. As such, roots are considered a central component of plant productivity (i.e., biomass production). However, the relative inaccessibility of the root system has caused root research to lag behind research into shoot development, physiology, and architecture.

Currently, the worldwide root research community is growing and becoming more active than ever. In the past few years, researchers have combined the latest technologies to image and quantify root systems (e.g., digital photography, x-ray computed tomography, transparent soils, high-throughput 3D reconstructions, or fluorescence-based imaging systems), generating an exciting landscape of new research strategies. In addition, recent collaborative efforts have led to the design of a common language for the description and storage of root architecture information. These technological innovations and robust standards have ushered in a new era in root research, namely, root phenomics. Phenomics is the scientific discipline concerned with the measure and study of phenotypes (physical form and function). Here, we will review the most recent advances in the phenomics of root system architecture and provide a comprehensive introduction to computational approaches that are becoming the new standards in root research.

ROOT SYSTEM ARCHITECTURE FORMATION

Root system development is an iterative process based on the repetition of four fundamental processes: (1) the production of roots, (2) their growth, (3) their growth direction in the soil domain (tropisms), and (4) their capacity to produce other roots (branching).

The first organ to emerge from the seed is the primary (or tap) root. The primary root grows and changes direction in the soil in response to various stimuli (e.g., gravity, moisture, or mechanical impedance). After a certain time, it forms branches (or lateral roots); the primary root gives rise to secondary roots, which give rise to tertiary roots etc. These branch roots are known as “higher order” roots. For a given species (or genotype), lateral roots emerge from their parent at a fixed time interval. As a consequence, the distance between two lateral roots (or interbranch distance) is a function of that time interval and of the parent root’s growth rate. The newly formed lateral roots emerge at a given angle with respect to their parent roots, called the insertion angle.

Once emerged, laterals extend into the soil and have the ability to change directions. Lateral roots can also form higher order branches, often with a different density than their bearing parent. This leads to the formation of a complex tree-like structure described as the root system architecture. A direct consequence of these dynamics is that the root system as a whole is composed of a large range of root ages, types, and developmental stages, which leads to a great heterogeneity in functional properties within each root system.

Monocots and dicots have distinct root system architectures. In dicot root systems, the primary root and first order laterals generally form the backbone of the structure. In addition, some dicot species form basal roots that originate from the hypocotyl and can represent an important proportion of the total root system. Monocot root systems can also form basal roots, also known as seminal roots. In addition to the primary and seminal roots, monocots usually form nodal roots (also known as adventitious or shoot-borne roots) that originate from the stem. As a monocot plant develops, nodal roots emerge from successive shoot nodes, both from the main stem and from the tillers (shoot branches, if present). Through this process, in monocot root systems, nodal roots generally constitute the majority of first order (or axile) roots. Adventitious roots are also observed in some dicot species, but in a less systematic fashion. Nodal and seminal roots follow roughly the same developmental program as the primary root, although, in some species, some specific genetic determinisms have been identified. The primary and basal (seminal) roots are sometimes referred to as the embryonic root system, while the nodal roots are referred to as the postembryonic root system.

The second difference between monocots and dicots is that dicot roots undergo secondary growth, but monocot roots do not. Secondary growth involves cell divisions in mature root tissues that leads to a thickening of the older roots in the root system, sometimes accompanied by the deposition of water-impermeable suberin. The absence of secondary growth in monocots limits their water transport capacity through the xylem because there is no increase in size of the xylem vessels with increasing numbers of lateral branches. That limitation is overcome by the continuous production of new nodal roots that tend to be thicker than their predecessors.

The effect of the different processes underlying root system formation on the overall architecture can be explored using the online version of the model CRootBox (https://plantmodelling.shinyapps.io/shinyRootBox/).

WHERE ARE THE SOIL RESOURCES, WHERE ARE THE ROOTS, AND WHAT ARE THEY DOING?

The role roots play in acquiring soil resources cannot be explained without first describing soil itself. Soil originates mostly from the
water uptake in roots is a complex process

Water uptake by the plant is a passive process, driven by differences in water potential along the soil, plant and atmosphere continuum. Water flows from areas of high potential (wet, low osmotic content) to low potential (dry, high osmotic content). Water evaporates from inside of the leaves to air through pores called stomata. This process, called transpiration, creates a suction that pulls water from the soil, into the roots, up the stem, and out the leaves. From the plant’s perspective, water uptake is actively regulated at multiple sites, both in the shoot and in roots. At the root level, the uptake capacity of a root segment (a short section of root) is influenced by its radial (from the soil to the xylem, across the root’s width) and axial (along its length) hydraulic conductivities.

The radial conductivity is mainly influenced by the root diameter, or the number of cell layers water has to cross before reaching the xylem poles, the presence of hydrophobic barriers (endodermis and exodermis), and the presence and activation of aquaporins, a large family of water channel proteins. Root diameter is usually variable among different root types and, for dicots, increases as the root ages due to radial growth. Hydrophobic barriers are tissue-specific secondary structures that impede the passage of water through the apoplast (the space between cells), forcing it to flow through the symplast (the space within cells). Recently, these barriers have been shown to form dynamically and degrade in response to environmental stresses. Aquaporins greatly facilitate the radial movement of water across roots by providing channels for water movement across membranes. Knocking down the production of certain aquaporins can reduce the uptake capacity of a plant by 60%. Aquaporins are not expressed uniformly within the root system, but rather their abundance depends on the root type, the root age, and the surrounding soil conditions. The root radial conductivity is then the combination of these three factors: root diameter, hydrophobic barriers, and aquaporins.

The axial conductivity, on the other hand, is mainly a function of xylem development, which is also highly related to root age, root type, and root diameter. Generally, thicker and older roots have larger and more numerous xylem poles, leading to a greater capacity to transport water.

Putting these concepts together at the root system level reveals a complex map of heterogeneous local water and nutrient uptake capacity and demonstrates that a great diversity of functional properties can be found within a single root system. Understanding at the plant level the functional importance of such diversity requires precise quantification of the root system architecture. Metrics describing the root system as a whole (that is, as a single object instead of a population of smaller structures) have been used for decades and are undoubtedly useful in many situations, but fail to capture the intrinsic richness of a root system structure and functions. Here, we describe tools that allow the heterogeneity of root system to be quantified.

How to describe root system architecture?

Phenes

Root systems are complex objects that are composed of thousands of elemental building blocks assembled in a highly organized network, and it is useful when describing root systems to introduce the concept of a “phene.” The entire physical description of an organism, or an individual organ such as a root system, is referred to as its phenotype. Phenes are defined as individually measurable components of the phenotype; phene is to gene as phenotype is to genotype. Phenes are represented by various measurements, similar to the representation of a physical gene as a DNA sequence written in letters. Phenes are inherently
“fuzzy,” in the sense they are represented by human measurements, under single-gene or multiple-gene control, influenced by the environment, and possibly created from or influenced by other phenes. The word phene has been used almost as long as the word gene and replaces the common use of the word “trait,” which can be used ambiguously and which is not a scientific term. (For those interested in semantics, consider that the definitions of biological terms such as gene and species have been continually updated throughout history and remain controversial.) We have found that a phene-based approach encourages exploration of phenotypes in new ways and believe the language of phenomics needs popularizing to facilitate understanding plant phenotypes.

Here, we describe root system phenes in four categories. The **geometry** of a root system describes the position of its root segments in space. The shape and form of individual segments are described by their **morphology**. How these segments are connected to each other to form a branched structure is described by the **topology** of the root system. Finally, the growth and development of the root system over time is described using **dynamic** phenes. In the following descriptions, we will focus on image-based approaches, as these have gained a lot of interest of the past few years. However, many of these phenes could be measured manually.

### Root System Geometry

The geometry of a root system describes the position of all the individual root segments. The simplest geometric features, and the ones that are probably the most widely used in root research, are the maximal depth and width of the root system. While these two variables give a good indication about the overall exploration, they give little information regarding the position of the root segments in space, nor their distribution within the root-system volume. This is usually done by extracting the distribution of the root segments along a vertical profile or by using descriptors of the root system shape, such as the convex hull area (the area taken up by the entire root system if a boundary is drawn around its edges).

Computing geometric features requires knowledge of the position of every root segment in the system, or at least the segments at the edge of the explored volume, but not the connections and relationship among these segments. However, once the root system is removed from its growing medium, the position of some roots, especially the finer ones, are likely to change, modifying its geometric features.

Geometric phenes have been shown, in many occasions, to be related to important root functions, such as water or nutrient uptake. For instance, some authors have observed that in wheat (*Triticum aestivum*), an increase in depth of only a few centimeters leads to substantial increases in yield. Geometric phenes have also been shown to be relatively good proxies for some fundamental processes. Depth is likely to be closely related to the primary root growth and width has been shown to be correlated with the lateral (or adventitious) root insertion angle and gravitropism. However, such correlations should be used with caution as similar geometric outputs (e.g., the same width) could be achieved by very different root systems. On the other hand, two root systems of the same genotype, grown in identical soil conditions might exhibit large variation in their geometric properties due to difference in local soil conditions (e.g., presence of rocks or wormholes).

### Root System Morphology

Morphological phenes describe the shape of individual roots or root segments. They include root segment diameter, root length, or local root orientation. In contrast to geometric features, morphological phenes do not require having information about every root. Similar to a researcher sampling several individual plants within a field, one could sample individual roots within the root system. Treating the root system as a population of roots makes it possible to obtain robust morphological data. Measuring the root diameter of 20 roots is often enough to have an accurate representation of the mean root diameter. However, one has to be careful, as such an approach only will work for supposedly homogeneous samples. For example, for diameter estimation, measurements should be taken only for the same class of root, and due to secondary thickening in dicots, used for monocots only.

Morphological features have also been shown to influence many plant processes. Increased root diameter negatively influences radial water flow and increases the cost of the root system because thicker roots are more expensive to construct and require more energy to maintain (known as “maintenance respiration”). Due to differences in nutrient mobility in soil, long lateral roots have been shown to be beneficial for water and nitrate acquisition, while short lateral roots with greater branching density are thought to be better for phosphate uptake.

### Root System Topology

The topology of a root system describes the connections and relations among its constituent roots. For a seedling, it is only necessary to identify the primary root and its laterals, which is usually a straightforward image analysis problem. However, for more complex (older) root systems, defining the topology consists of reconnecting all roots to their parents, which can be a long and tedious process. As a result, topological features are difficult to obtain from mature root systems.

Once the topology is established, rich information related to the root system development is available. The number of lateral roots, the inter-branch distance and the lateral insertion angle are examples of phenes that can be computed from the topology. By combining topological and morphological phenes, it becomes possible to compare the properties of different root types.

Another approach to root system topology is the use of a mathematical tree, which allows obtaining a condensed history of morphogenesis rather than only the final outcomes, such as total root length. For instance, the altitude of a root system is the length of the path between the base of the root system and the furthest root segment (in terms of numbers of connections, not Cartesian length). The magnitude of a root system is the sum of the length of the paths between the base of the root and each root apex. These metrics have successfully been used to quantify root system topology and to discriminate among species, genotypes, and soil treatments.

### Root System Dynamics

Finally, dynamic phenes can describe plant developmental processes. Growth rates, lateral emergence rate, and gravitropism are some example of dynamic phenes. Dynamic phenes provide
information about the age distribution of the different root segments. As discussed earlier, the age of a root segment is often linked to the functional properties of the segment and is therefore an extremely valuable parameter to acquire.

Retrieving dynamic information requires geometric, morphological, or topological data about the same root system at a minimum of two time points (but the more time points the better). Like for the geometric features, computing dynamic phenes does not require having information about all the roots. Following a subset of roots across the time series is enough to have a representative vision of the dynamic phenes. The main difficulty in the acquisition of these metrics lies in the fact that the data acquisition method cannot be destructive because data from different time points are needed. This difficulty could be overcome by using different plants for each time point and combining the results for different genotype-by-environment combinations.

Fortunately, relationships among static and dynamic phenes have been shown for various species. For instance, the time between the formation of a lateral root primordia and its emergence is, for several species, constant. The distance between the first visible lateral root and the apex of the bearing primary root (length of apical unbranched zone [LAUZ]) is therefore dependent on the root growth rate. Such simple relationships are useful to infer dynamic phenes from morphological ones when multiple time point sampling is not possible (e.g., in the field). Another well-documented relationship is between the diameter of a root apex and its growth rate. Larger roots have larger meristems and, therefore, a higher potential growth rate. However, this potential growth rate is often not reached, due to various endogenous or exogenous stresses such as soil impedance, structural carbon shortage, or nutrient deprivation.

Dynamic traits can be used to calibrate root architectural models such as OpenSimRoot (https://rootmodels.github.io), CRootBox (https://plant-root-soil-interactions-modelling.github.io/CRootBox), RootMap, or ArchiSimple. These models, once properly calibrated, can then be used to explore functional properties of given architecture, therefore extending the scope of the structural analysis.

**Root System Markup Language**

Many root architecture-related software tools are used by the scientific community. These tools range from image analysis, to data analysis, to root modeling. However, until recently, data exchange between the different tools was not possible due to incompatible root architecture information (different file format and formalisms were used). In 2015, the Root System Markup Language (RSML) was designed as a common storage and exchange format for root system architecture information.

The RSML format is based on the XML formalism, a format that is widely used in computing and is supported by many coding languages. Without going into technical details, the RSML format allows the storage of the topology, morphology, and geometry of a given root system. So far, the formalism has been implemented in a dozen image analysis tools and is also used by data analysis pipelines and modeling platforms (either as modeling input or outputs). More information about the compatible tools and the RSML formalism can be found at http://rootsystemml.github.io/.

**HOW TO ACQUIRE ROOT ARCHITECTURE DATA?**

The underground nature of roots makes them difficult to observe. Direct measurements of roots in field conditions generally require removal of soil to observe roots, which results in the death of the plant, the loss of root material, and the loss of geometric information. Newer methods in the lab or greenhouse allow for faster and more detailed measurements of root phenes; however, the relationship of phenes measured in controlled conditions with phenes in natural or agricultural conditions is largely unknown. Over the past century, researchers have developed several plant growth platforms that enable the observation of root system architectures.

John E. Weaver (1884–1966) was among the earliest scientists to document the growth and development of entire root systems of crops and wild plants. The basic method employed was digging a trench next to growing plants to create a smooth surface on which the roots could be observed. Lore Kutschera (1917–2008) and Monika Sobotik spent much of their lives using this technique to expose and draw root systems, producing an invaluable library of root system architecture drawings. Nowadays, the trench technique is mainly used to quantify the amount of roots across a 2D profile, usually by placing a wide mesh (2–5 cm) on the surface of the profile and either counting the number of visible roots or, more simply, noting the presence of the roots in each cell of the grid. While the trench method allows for a precise in situ observation of roots grown in the field, it is very laborious and slow.

Aside from the trench technique, field researchers have been using excavation techniques to sample and phenotype roots. Soil coring involves inserting a metal cylinder down into the soil to obtain a soil core (like the ice cores used in climate research). Traditionally, the cylinder was inserted with hammers, but recently hydraulic rigs mounted to tractors or other vehicles have made soil coring much easier. The soil core is usually divided into segments of the same length, and each segment is washed over a screen to collect all the roots. These roots are scanned on a flatbed scanner for subsequent length measurements. The roots can also be dried and weighed, or simply counted. Core sampling in the field allows researchers to estimate root length density along the depth profile. While the method provides only limited information about the root system architecture, it has the advantage that it can be relatively well automated.

More recently, a different excavation technique was introduced into widespread usage within the scientific community. Root crown phenotyping, or shovelomics, is simple: Using a regular shovel, the root crown (the upper part of the root system, which is directly attached to the shoot) is excavated, washed, and stored for further scoring. The root crown can be thought of as the backbone of the root system and, even though incomplete, it determines the overall placement of roots in soil. Measurements of the number of roots, their angles, and lengths provide a wealth of data. Compared with the trench method, this technique has the advantage of being fast and it can be automated to a certain extent.

However, both coring and shovelomics are destructive techniques. Other methods are needed for observing root growth over time. Field rhizotrons are an improved version of trenches. In this method, a trench is dug, a glass window is placed tightly over the vertical cut plane, and a roof is installed over the pit. Researchers can come back repeatedly to trace and measure roots.
Mini-rhizotrons are transparent tubes that are inserted into soil down to several meters in depth. A customized camera can be inserted into the tubes to image the soil and the roots around the tube. It is possible to observe the initiation, growth, and turnover of individual roots over long periods of time using rhizotrons or mini-rhizotrons.

As stated above, the major limitations of field-based techniques are the low throughput (small numbers of samples per unit time) and the influence of local environmental variations on root architecture. In order to increase the throughput of data acquisition and reduce the effects of environmental variability, researchers have developed a whole range of plant growth platforms. These setups can be classified according to the “naturalness” of the root environment, ranging from soil to highly artificial media, or even no solid media at all.

One of the most commonly used platforms in root research is the rhizobox, based upon the principles of the field rhizotron discussed above. Rhizoboxes are flat, rectangular containers (usually a few millimeters or centimeters thick) that can vary in size and design, with at least one transparent face acting as an observation window to record and quantify root system growth and development. Often, rhizoboxes are maintained at an angle which forces the roots to grow along the glass. The window is covered except when measurements are made. One of the main issues with rhizoboxes, as with other rhizotron-based methods, remains the distinction between the root and the soil or growth media (this is especially true for thin roots). Recently, a better distinction between the root and the soil has been enabled by the use of transgenic plants that constitutively express luciferase. Luciferase is an enzyme that produces bioluminescence (light) when provided with the appropriate substrate, luciferin; this system is described as GLO-roots. Rhizotron-like growth platforms can vary considerably, as exemplified recently by the use of transparent pots with plants grown at their outer surface, therefore forming circular rhizoboxes.

Hydroponics and aeroponics allow for the cultivation of plants without solid substrate, which makes the roots completely visible in 3D and easy to study over time. Both systems can be useful for several reasons. First, nutrients can be manipulated easily and changed over time so their influence on root growth and physiology can be studied. Second, plants can be temporarily removed from the growing setup to be imaged or sampled without damaging the root system. In hydroponics, roots are submerged into a nutrient solution composed of water with all the nutrients needed for the plant growth. Usually the solution is oxygenated using bubbler like those used in an aquarium. However, submersion in water can substantially change root system architecture. In aeroponics, roots are suspended in the air in a closed container where nutrient solution is misted periodically. Aeroponic systems have the advantage of not submerging roots in water and enabling greater access to oxygen. It should be noted that when using hydroponic and aeroponic systems, root anatomical differences can be observed. For instance, formation of an exodermis (suberization of the external layer of the cortex) occurs in soil and aeroponics, but not in hydroponics.

Other clear media have been used to grow roots, such as gellan gum, agar (in Petri dishes), and transparent “soils.” These have the advantage of providing physical substrate while also allowing the roots to be freely observable. On the other hand, they are still not “natural.”

X-ray computed tomography (CT) and magnetic resonance imaging (MRI) are two techniques that enable the imaging of root systems grown in more or less natural soil, without destroying the plant and therefore allowing for time-series measurements. Both systems rely on different physical processes that influence the type of acquired data. With x-ray CT both roots and soil are imaged and custom-made software tools are needed to segment the roots. On the other hand, MRI can be adapted to image only the roots in such a way that no segmentation (separation of root data from background [soil] data) is required. However, in many situations, a quantification of soil properties is desirable. While these techniques open new avenues in soil-root research, they remain relatively low throughput and, most importantly, have a very high price tag.

In summary, there are many ways to grow and observe roots. Methods should be chosen that balance experimental goals such as detail, time series, similarity to field conditions, cost, and ease of use.

**HOW TO ANALYZE ROOT SYSTEM ARCHITECTURE IMAGES**

Similarly to root image acquisition, the analysis of root images often results in a trade-off between the complexity of the images, the level of detail of the extracted data and the level of automation of the software tool. Software tools analyzing root images have to deal with two specific issues related to the root system structure: overlapping roots (roots growing in the same direction, on top of each other) and crossing roots (roots that cross while growing in different directions). Both situations make it difficult to automatically and accurately identify branching patterns in extensive root systems. For images of young root systems with a low level of overlaps and crossings, the complete root architecture can usually be extracted automatically. However, as the complexity increases, automated algorithms are more prone to failure. Therefore, when dealing with complex root images, researchers face two options: They can either move to semiautomated solutions or use aggregated metrics.

Semiautomated root tracing tools were designed to streamline the manual root tracing process. They can be divided in two main categories: correction-based or click-based. Correction-based tools feature an automated root detection algorithm that is followed by a correction step (whose user-friendliness varies between tools). Click-based tools are designed to streamline the tracing of individual roots by the users (one click is usually sufficient to trace one root). If time is not a restriction, they can be used for the analysis of very complex root structures. However, for large scale studies, in which thousands of images have to be analyzed, the amount of time required might be prohibitive.

In situations where very large numbers of images need to be analyzed, or when root images become too complex to be analyzed manually even with semiautomated tools, researchers usually turn toward fully automated tools designed to extract a mixture of geometric and morphological information. While these tools give fewer details of the root system architecture, they have been used particularly in genetic studies to address the effect of genotype on phenotype on the root system development.

The choice of the analysis tool must be in line with the considered scientific question. For instance, when studying the effect of a specific compound on branching densities, it is necessary to acquire detailed information about root system architecture. On the other hand, if one is interested in the evolution of the root:shoot surface ratio, the projected root surface, which can be extracted
automatically, is likely to be enough. Available options concerning root image analysis tools, as well as the growing and imaging setup, must therefore carefully be considered before starting the experiment. A directory of available root image analysis tools and their characteristics can be found on the plant image analysis website (http://www.plant-image-analysis.org).

HOW TO ANALYZE ROOT SYSTEM ARCHITECTURE DATA

Once root system architecture information has been extracted from a root system, the last step is to analyze and interpret the data. If the full root system architecture was extracted, many types of analyses can be performed. Again, as stated earlier, the analysis should be carefully chosen to match the experimental needs.

The easiest way to analyze root data is to aggregate it at the root system level. Many types of data can be aggregated at the root system level, or by types of roots, such as the total root length, the number of lateral roots, or the mean lateral insertion angle. Once the data have been aggregated, they can either be compared one phene at a time between the different genotypes or conditions, or they can be analyzed simultaneously using multivariate analysis.

Common goals of root research are to distinguish genotypes or to show differences based on a condition, such as drought or low soil fertility. In these cases, categories are created and compared and the suitable statistical technique is analysis of variance, commonly called ANOVA. ANOVA evaluates the variation within and among these categories with the idea that greater variation among groups than within groups implies real differences among those groups.

In other cases, a desire to relate the variation in one root phene to another root phene, or to variation in plant performance as measured by plant mass or yield, requires the use of linear regression techniques. Simple ANOVA and linear regressions can be performed in Excel and other spreadsheet software, but learning the computer language R (open-source) is recommended for these and more advanced techniques.

Principal component analysis (PCA) is a statistical technique that aims at reducing the number of phenes in a data set by capturing the greatest amount of variation using linear combinations of all the measurements called principal components. Though the underlying matrix algebra is complicated, the results may reveal combinations of phenes that can be interpreted in meaningful ways and reduce the complexity of the data set. Principle components are generally ranked by the fraction of the total variance that they account for individually. Essentially, these components can convert many measurements into one new property, called a score, which can be used for subsequent analysis just as the original phenes were used. In root data, we commonly find measures related to root system size and complexity loading onto one component and perhaps measures related to width and angles onto another. That several related phenes load onto a principal component implies possible shared developmental and genetic processes.

CONCLUSION

Understanding how roots forage for soil resources is imperative for closing the yield gap (the difference between theoretical and actual yields) while the global population grows and climate changes. Therefore, acquiring root data quickly and accurately is an important challenge. This challenge is being met using various imaging approaches coupled to image analysis, borrowing from the fields of computer vision and machine learning. The phenomics of root system architecture will allow the integration of beneficial phene states in elite crop varieties to feed the world.

RECOMMENDED READING

(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

Introduction

Root System Architecture

Root-Soil Interactions


Root Phenotyping


Root Image Analysis


Root Data Analysis


Root Models


