

# How can we harness quantitative genetic variation in crop root systems for agricultural improvement?

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**Abstract** Root systems are a black box obscuring a comprehensive understanding of plant function, from the ecosystem scale down to the individual. In particular, a lack of knowledge about the genetic mechanisms and environmental effects that condition root system growth hinders our ability to develop the next generation of crop plants for improved agricultural productivity and sustainability. We

discuss how the methods and metrics we use to quantify root systems can affect our ability to understand them, how we can bridge knowledge gaps and accelerate the derivation of structure-function relationships for roots, and why a detailed mechanistic understanding of root growth and function will be important for future agricultural gains.

**Keywords:** Architecture; genetics; imaging; quantification; root  
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Invited Expert Review

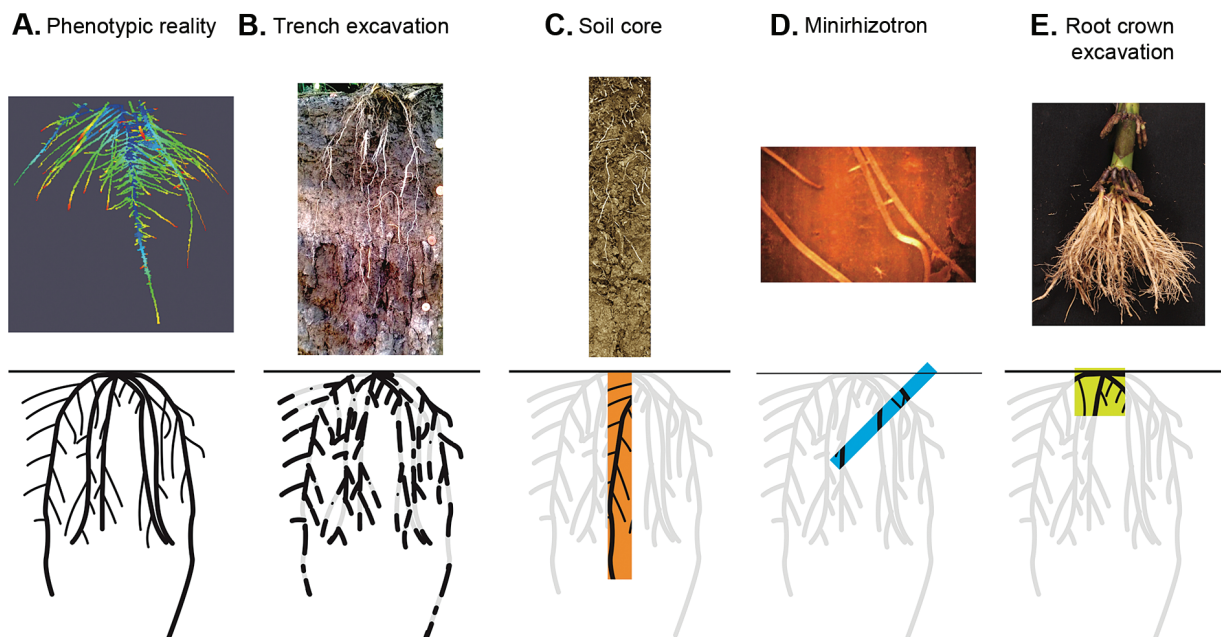
## INTRODUCTION

Despite long-term scientific interest, the genetic and functional basis of root architecture remains obscured, largely due to the difficulties in imaging and quantifying roots and their interactions with the environment. To date, only two genes have been reported that control root architecture quantitative trait loci (QTL) in a crop plant, Deeper Rooting 1 (*Dro1*) and phosphorous-starvation tolerance 1 (*Pstol1*) (Gamuyao et al. 2012; Uga et al. 2013). Both can confer increased rice yields under drought and low phosphorous, respectively, underscoring the enormous potential of root systems to boost and stabilize crop yields under stress. But these successes were the product of intensive mapping and introgression efforts that began years earlier (Wissuwa et al. 2002; Uga et al. 2011), and represent only a tiny fraction of the earth's biodiversity for root traits. Given projected global demand for crop products and the negative consequences of climate change on agriculture, the pace of discovery and translation must dramatically increase (Tilman et al. 2002; Fedoroff et al. 2010).

Leveraging the vast amount of genetic variation identified from high-throughput sequencing technologies will require comparably scaled innovations in plant phenotyping tools (Furbank and Tester 2011; USDANSF 2011). Image-based phenotyping has enabled relatively high-throughput and accurate measurements of roots, but despite an expansive list of new and promising methods (Lobet et al. 2013), inherent

tradeoffs of each have greatly limited their individual power to resolve the underlying genetics. The spectrum of tradeoffs usually involves: (i) throughput allowing analysis of large populations; (ii) realism of growth conditions (e.g., controlled environments vs. field conditions); and (iii) information content of the measurements (e.g., whole vs. parts of the root system, young vs. mature plants, 2D vs. 3D, temporal resolution and functional capacity). Genetically encoded differences aside, the oft-cited phenotypic plasticity of roots coupled with the variability of climate, soil and biotic interactions pose additional challenges to the study of roots in natural environments. While high-throughput, non-destructive, whole-root system phenotyping in the field remains a key conceptual goal, this reality is at best on the distant horizon.

Since there is not a “one size fits all” method for root phenotyping, any given data set can only represent some limited aspect of phenotypic reality (Figure 1) (Bildler et al. 2009; Houle et al. 2010; Granier and Vile 2014; Chitwood and Topp 2015). It is then important to consider how we might bridge these information gaps when addressing a particular question in our research. In this review we will discuss how our understanding of root architecture and its genetic basis is shaped in part by how, when and what we measure; how we can identify and quantify the functional attributes of root systems; and how we can use this information to further our understanding of whole plant function and root-environment interactions for agricultural benefit.



**Figure 1. The range of root architecture-related information captured by common field phenotyping methods**

Top row portrays data indicative of each method. Bottom row portrays a 2D representation of the relative information captured by each method. **(A)** Phenotypic reality encompasses the entire 3D root structure through time (4D), including local root growth. These data cannot currently be captured in the field, but can be approached for young plants in controlled environments. Shown here is the time function generated by analyzing sequential 3D reconstructions representing seedling root growth, where warmer colors indicate later time points. The growth of each root and its contribution to the geometry and topology of the system architecture can be explicitly known in this way (Symonova et al. 2015). **(B)** Trench excavations are the gold standard for field root phenotyping since, given sufficient labor, they can capture a relatively comprehensive 2D view of naturally occurring architecture compared to other methods. The related and more sophisticated slab or monolith methods can theoretically extract entire root systems in 3D; however, this information is not easily quantified, and due to the time and effort involved, the method is rarely used in modern research. **(C)** Soil cores are a primary way to capture information relating root densities to depth for a single time point in an essentially 1D sample, although multiple samples can be taken to infer coarse 3D geometry, but not topology. **(D)** Minirhizotrons incorporate elements of time and depth to root density information, but are also essentially 1D and limited to those roots that grow on the surface of the transparent tube. Similar to soil cores, they can be combined *en masse* to infer coarse 3D geometry. **(E)** Root crown excavations, or “shovelomics”, capture an information-rich area of the root system that contains the origins of the primary root axes. However, the roots are typically so dense that manual or optical methods can only extract a portion of the geometric and topological information contained in the sample. Other methods, such as X-ray computed tomography (see Figure 2), can improve this.

## HOW DO WE KNOW WHAT WE KNOW ABOUT ROOT PHENOTYPES?

Much of the foundation of what we know about the shape, size and architecture of root systems growing in natural and agricultural environments comes from John E. Weaver and colleagues. Over decades of work, they refined methods for excavating and documenting root systems to produce elegant two-dimensional reconstructions (Weaver et al. 1922; Weaver 1926; Sperry 1935; Weaver and Voigt 1950; Kutschera 1960; Bohm 1979). Traditional excavations and related approaches such as soil coring and minirhizotron tubes are still the state of the art for field root phenotyping (Trachsel et al. 2010; Maeght et al. 2013; Wasson et al. 2014), but require significant time, capital and human resources. Even so the resultant data typically can only estimate limited aspects of the root phenotype, and the overall root structure

must therefore be cautiously inferred (Figure 1) (Heeraman and Juma 1993; Kücke et al. 1995). With the goal of identifying the genetic basis of root phenotypes in mind, new higher-throughput, lower-effort approaches that capture accurate information about root architecture and function are warranted.

Numerous root phenotyping platforms have recently been developed as novel or more powerful takes on existing root and rhizosphere methodologies, as detailed in several recent review papers (de Dorlodot et al. 2007; Neumann et al. 2009; Maeght et al. 2013; Meister et al. 2014; Downie et al. 2015; Kuijken et al. 2015). One important example is shovelomics, which focuses on the high-information content of easily accessed root crowns using standardized methods for manually estimating developmental and architectural traits (Trachsel et al. 2010). With enough labor this method is scalable to very high throughput (1,000's of plants at  $\approx 100$

plants per person, per day), but still requires trained eyes and extracts only a fraction of the possible information contained in the sample. However, the recent adoption of image-based phenotyping for excavated root crowns provides a much more information-rich data set from the same samples (Grift et al. 2011; Bucksch et al. 2014; Colombi et al. 2015; Das et al. 2015), and one software package is now publically available on a widely accessible computing resource (Das et al. 2015). Indeed, a key aspect of many modern approaches is the incorporation of image-based quantification methods (Spalding and Miller 2013), and to some extent, automation and robotics (Nagel et al. 2012; Subramanian et al. 2012; Slovak et al. 2014). Image-based analysis has the enormous potential to standardize the accuracy and precision of root phenotyping through defined algorithms that are vetted against ground truths, and to simultaneously broaden the metrics we use to characterize roots, which will improve our power to map genotype to phenotype. Evidence for this idea was highlighted by Houle et al. (2010) (from the work of Liu et al. 2010): Seven loci were known to control human eye color, which is traditionally classified in either blue, green-hazel or brown categories. However, quantification of continuous hue and saturation space from digital images of eyes identified additional axes of variation and three additional loci controlling eye color (Liu et al. 2010). This variation was hidden from previous studies, due solely to how the eye color phenotype was measured.

#### **Our ability to correlate laboratory and field data is conditioned by the information content of what we measure and how well we measure it**

As we transition to similar new paradigms in our understanding of root phenotypes, we can question how the limitations in our ability to comprehensively measure roots affects what we “know” about them (Pierret et al. 2005). It has been pointed out in numerous works (Heeraman and Juma 1993; Wissuwa et al. 2009; Wasson et al. 2012; Shrestha et al. 2014) that there can be little correlation between root phenotypes collected in controlled environments or fields, but to what extent does how and which measurements we make affect these conclusions? As a thought experiment we can consider a trait such total root length (TRL). TRL may be highly heritable as measured from seedlings in a lab-based imaging system, but low when measured by excavation or coring of mature plants with identical genotypes in some field setting. There are a large number of developmental, physiological and environmental interactions that could contribute to this difference, but it is also true that from 2D images or 3D models we can explicitly measure the length (and much more) of the entire root system, whereas from the field we can only extrapolate the total root length from the portion of the roots that were recovered and accurately measured after sampling and washing (Figure 1). Our ability to consistently repeat the controlled environment experiment with great precision and accuracy is high, but may be quite low in the field, especially among the multitude of possible field settings. If we could measure roots of mature plants in any field setting with similar efficacy as in a lab, we might find a stronger heritable component of the phenotype, and a much higher correspondence between controlled environment and field data, at least for some cases.

Despite biological and methodological complexities, there are several lines of evidence that suggest strong lab to field correspondences can be drawn for root phenotypes that relate to agricultural productivity (Tuberosa et al. 2002a; Khowaja et al. 2009; Wishart et al. 2012; Hufnagel et al. 2014). The identification of the *Dro1* QTL in rice was identified through low-tech “basket assays” (Uga et al. 2011), and the subsequent cloning and characterization of gene function relied on Petri-dish based seedling root gravitropism measures (Uga et al. 2013). Similarly, the phenotypic effects of *Pstol1* underlying the phosphorus uptake 1 (*PUP1*) QTL in rice were eventually characterized from controlled environment pot and molecular assays (Gamuyao et al. 2012). It is easily envisioned that either *Dro1* or *Pstol1* could have been identified *de novo* via modern high-throughput root screens that measure cellular growth dynamics in real time (exemplified in Meijón et al. 2014). In related work, direct genetic linkages were drawn between causative single nucleotide polymorphisms (SNPs) in *Pstol1* sorghum homologs for quantitative variations in seedling 3D root architecture measured from gels and with yield in low-phosphorous field settings (Hufnagel et al. 2014). While these successes may to an extent reflect the large effect sizes on the quantitative traits used for characterization, there is reason for optimism that subtler phenotypes that are robust across lab and field studies may be captured as our phenotyping and analytic methods improve.

#### **New and more nuanced phenotypes will contribute to our understanding of root architecture and its genetic basis**

Apart from directly comparable lab to field measurements such as TRL, there are aspects of root phenotype that are currently not possible to measure using field-based or manual methods. One example is circumnutation, the helical growth of roots relative to the growth environment, which may help us to understand endogenous strategies plants use for resource foraging. Measuring circumnutation requires imaging that captures both temporal and 3D space (e.g., Clark et al. 2011), and thus precludes most field and even lab-based approaches. The ability to resolve the growth of each individual root in the context of the entire root system also promises to advance our understanding of the relationships between local and systemic growth patterns, and their conditioning by internal and external signals (Moore et al. 2013; Symonova et al. 2015). With these tools we can begin to answer questions about how local growth behavior at each root tip contributes to the overall root architecture, and how such information is processed system wide, which would provide valuable data for modelling. Similarly, as we develop finer-grained appreciation for the roles of specific root types in root function and environmental interactions (Clark et al. 2011; Chochois et al. 2015; Gutjahr et al. 2015; Yu et al. 2015), the context of how much and how well we measure will gain importance. The benefits for quantifying many aspects of root architecture have been borne out by studies employing machine learning to compare several genotypes of either rice (Iyer-Pascuzzi et al. 2010) or maize (Zurek et al. 2015). In both studies, an array of shape descriptors was used to quantify aspects of each root system, but the descriptors that delineated any given pair varied depending on the genotype and developmental time. Similar to human eye color (Liu et al.

2010), the ability to measure many potentially subtle differences in phenotype can have direct implications for our understanding of the genetic basis of root architecture – new large-effect QTLs were identified in a rice mapping population using a multivariate approach that were absent when queried with single univariate traits (Topp et al. 2013). In general, the more complex the system, the more the information content of our measurements is likely to be important for our comprehension of the effects of phenotype and its environmental and temporal control.

## HOW CAN WE USE A GROWING BODY OF KNOWLEDGE ABOUT ROOT ARCHITECTURE TO UNDERSTAND ROOT AND WHOLE PLANT FUNCTION?

As we are increasingly able to describe and quantify root architecture, so will we increasingly understand how plants function as integrated systems. Since the resources that roots forage are distributed heterogeneously in time and space, it stands to reason that numerous functional links should exist between root growth/architecture and the elemental, metabolic and physiological attributes of crop plants. Yet few of these key connections have been conclusively demonstrated. Spotlighting this black box, we have even a poor understanding of how root, soil and hydrological processes interact to drive the basic root function of water and nutrient uptake (Zarebanadkouki et al. 2014).

### Leveraging genetic resources to generate structure-function relationships

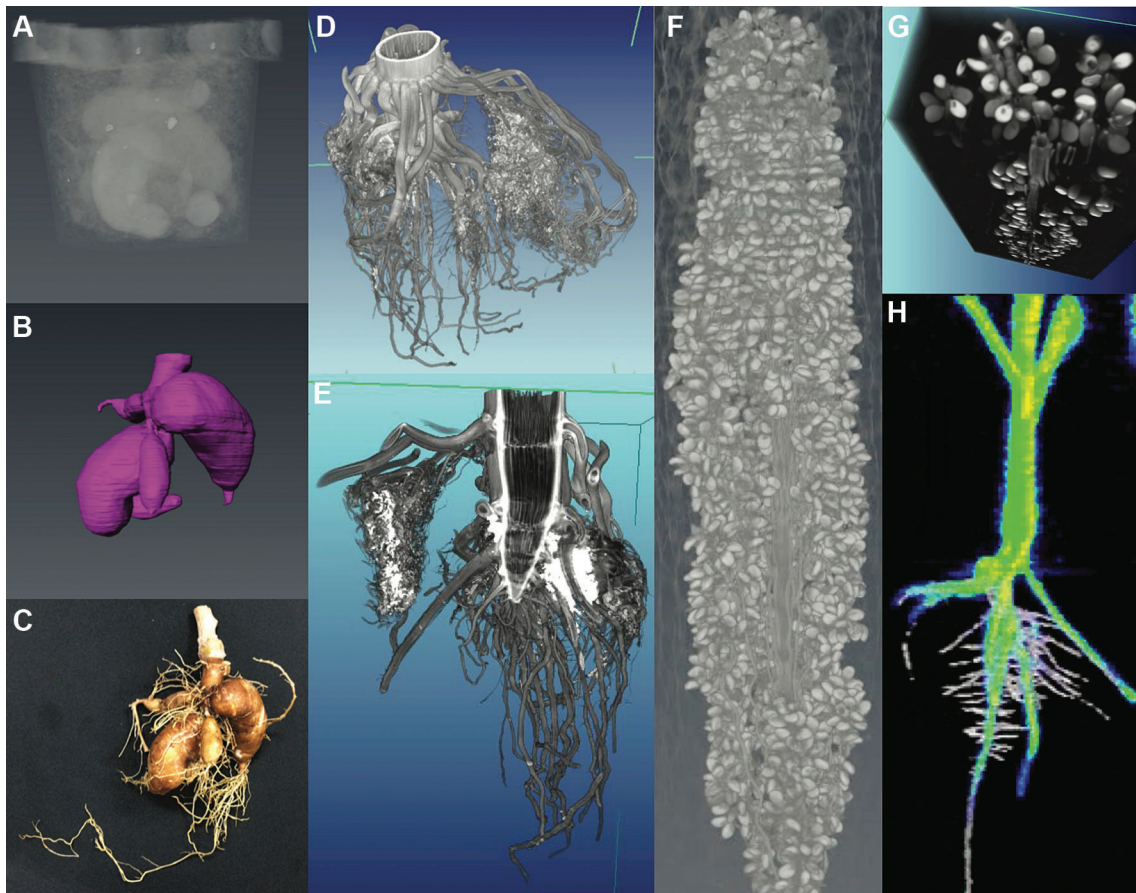
One way to connect structure and function is to start from germplasm with known contrasting agronomic qualities that are likely related to root architecture and growth, and work back to identify a genetic and/or mechanistic basis. Recent studies concerning the role of roots in planting density adaptations that have driven gains in US maize yields over the past 80 years (Hammer et al. 2009; York et al. 2015) have identified one such fertile research area. Using hybrids that span a historical continuum of maize breeding lines, York et al. (2015) uncovered specific architectural attributes that may have contributed to enhanced crop performance at high density. Employing a similar approach with populations bred solely and continuously for high yield at increasing plant density may provide a crucial resource to identify the genetic targets of this artificial selection process (Brekke et al. 2011a, 2011b). Similarly, the Illinois Long-Term Selection experiment could provide an opportunity to make functional links between nitrogen uptake and root architecture (Moose et al. 2004). Over more than 100 years of recurrent selection for (nitrogen-rich) protein content of seeds, the high-protein lines have acquired superior N-uptake capacity compared to low-protein lines (Uribelarrea et al. 2007). These changes are presumably related in part to adaptations of root architecture. Thus the development of multiple intermated recombinant inbred line populations should provide for “high-resolution” mapping of the genetic basis of nitrogen uptake and other traits related to strong, directed selection for seed protein content (Lucas et al. 2013).

### Combining above- and below-ground measurements will lead to a more comprehensive understanding of plant phenotype

Integrating shoot and root phenotyping in the same experiments will extend our ability to link roots to their functional attributes, especially when combined with powerful genetic resources. The combination of in-depth root analyses with the vast number of emerging high-throughput, high-information content aboveground phenotyping methods (e.g., Andrade-Sanchez et al. 2014), could greatly improve our understanding of whole-plant function. A major target in this area would be to identify fast aboveground measurements that robustly reflect root phenotypes. Although crop canopy temperature is often used as a coarse proxy for root function, these associations are typically weak and are not necessarily predictive outside of a given experiment (e.g., Wasson et al. 2014). One particularly intriguing combination is combined analysis of root traits with the elemental content, or ionome, of shoot tissues. Since non-carbon elements of plants are largely provided through the root system, links between root architecture and ionomes could highlight functional relationships with roots and soil conditions (Baxter and Dilkes 2012). Using high-throughput phenotyping, an *Arabidopsis* leaf ionome has been shown to effectively read out plant physiological status (Baxter et al. 2008), and maize seed ionomes were shown to be sensitive indicators of different plant growth environments (Baxter et al. 2014). Regardless of the approach, ground truthing methods that determine the accuracy of the associations with hi-fidelity, comprehensive above- and below-ground measurements will be critical.

### Advanced imaging tools can be used to integrate molecular, metabolic, physiological, and micro and macro morphological information in situ

The cutting edge of plant phenotyping technology is now providing otherwise unobtainable views of plant structure-function. The advent of fluorescence-based, genetically encoded biosensors offers a new paradigm for studying fundamental metabolic and physiological processes *in situ*, including the dynamics of primary metabolites, such as sugar compounds and hormones, that drive whole-plant physiology (Chen et al. 2010; Waadt et al. 2014). Imaging methods originally developed for medical and industrial purposes are also being used for plant imaging to great effect. Neutron imaging and X-ray tomography (XRT), which respectively use the heterogeneity in neutron or X-ray-attenuating properties of the target to form contrast images, have already generated advances in our understanding of plant structure-function relationships. Both can image roots in opaque substrates, including field soils (Figure 2). Neutron imaging is particularly well suited to quantify the dynamics of water uptake by root systems, as deuterated (heavy) water can be injected and directly tracked (Moradi et al. 2008; Warren et al. 2013; Zarebanadkouki et al. 2013; Zarebanadkouki et al. 2014). X-rays have been used for much longer to analyze root and soil structures and the interface between them. Analyses range from micro-scale interactions such as root-hair-rhizosphere and root-soil-particle interactions, to macro-scale interactions of root/ systems focused on root-root, and root-nutrient interactions at an architectural level (Pierret and Moran 1996;



**Figure 2. Advanced imaging technologies will help elucidate relationships between root architecture, physiology and function (A–C)** *In situ* X-ray computed tomography (XRT) analysis of cassava storage root growing in a ca. 3 inch opaque pot with soil substrate. (A) Raw XRT reconstruction. The scan took ca. 5 min at 110 micron resolution. (B) Segmented 3D root reconstruction, ready for quantitative analysis. Fine root structure was intentionally left out. (C) Excavated and washed root sample. (D) XRT reconstruction from maize root crown excavated from a field and washed (akin to panel E in Figure 1). The scan took ca. 2 min at 110 micron resolution. (E) A digital cross-section of the same root as in D showing internal morphological information. (F) XRT reconstruction of a sorghum inflorescence. The scan took <5 min at 114 micron resolution. Many features, such as seed number ( $n = 661$ ), were instantaneously quantified in the intact sample. (G) A digital cross-section of the inflorescence in F showing the complex branching architecture otherwise hidden from optical methods. (H) Combined optical projection tomography - positron emission tomography (OPT-PET) data set from a maize seedling pulse labelled with  $^{11}\text{CO}_2$  gas for 10 min. Color data are quantitative representations of the presence of carbon-containing photosynthates, where warmer colors equal more carbon. Greyscale information was generated from 3D optical imaging and represents the root morphology. Note that not all roots, or all parts of roots, co-localize with carbon signal because these data are a snapshot of dynamic carbon allocation patterns in the maize root.

Moran et al. 2000; Kaestner et al. 2006; McCully et al. 2010; Mooney et al. 2011; Metzner et al. 2015; Pfeifer et al. 2015). Additionally, XRT can be used to generate detailed information about whole-plant morphology, both internally and externally, at multiple spatial scales, either *in situ*, or after destructive sampling (Figure 2). Magnetic resonance imaging (MRI), which relies on the radio waves generated by physical interactions with magnetic fields to generate images, has also been effectively used to study root structure, growth dynamics, and water content *in situ* (Jahnke et al. 2009; Schulz et al. 2012; Metzner et al. 2015). Finally, positron emission tomography (PET) is a powerful and versatile functional imaging modality that can be used to study whole-plant dynamics in living specimens. PET generates high-

resolution images in real time by detecting characteristic emissions from tracers that incorporate high-energy radioisotopes. The tracers can be particular plant metabolites such as hormones and sugars, or raw materials such as carbon dioxide, water and nitrate, and introduced to the plant via direct application, photosynthesis (for  $\text{CO}_2$ ) or root uptake (Kiser et al. 2008; Suwa et al. 2008; Jahnke et al. 2009; Tsukamoto et al. 2009; Kanno et al. 2012; Lee et al. 2013; Wang et al. 2014; Karve et al. 2015; Pankiewicz et al. 2015). When combined with XRT, MRI or optical imaging, PET can directly measure structural-functional relationships (Figure 2) (Jahnke et al. 2009).

However, employing these advanced imaging technologies effectively for plant research faces numerous obstacles.

With the exception of X-ray computed tomography, which is finding its way into the hands of more and more plant researchers, most of these technologies require close partnerships with physicists at national labs or medical schools to conduct experiments. Much more investment in plant research and growth infrastructure at these institutions will be needed to incentivize highly productive relationships that can truly approach fundamental questions in plant biology in new ways. Regardless of where the information comes from, robust modeling frameworks that incorporate mathematical, statistical and signal processing theory will be absolutely essential to linking root architecture and function (Rengel et al. 2012; Granier and Vile 2014). Although there are many bridges to build among basic and applied research communities in order to assemble the right mix of expertise within a team or collaborating groups, the potential is enormous to reveal fundamental information about the ways root and shoot systems are integrated.

## WHAT ARE THE IMPLICATIONS FOR UNDERSTANDING THE GENETIC BASIS OF ROOT TRAITS VERSUS BREEDING FOR THEM DIRECTLY OR FOR YIELD IN TARGET ENVIRONMENTS?

Given the incredible difficulties in capturing, assessing and assigning function to root architecture traits, it is reasonable to ask if alternative methods will better achieve improved root systems for agriculture. Over the past century, strong artificial selection for aboveground traits combined with improved agricultural practices and inputs have greatly contributed to steadily rising crop yields in advanced farming systems (Duvick 2005; Ciampitti and Vyn 2012). During this time, improved root traits have undoubtedly, if indirectly, been selected for (e.g., Hammer et al. 2009; York et al. 2015). However, understanding the extent to which these improvements are limited to target breeding environments and resource-intensive management practices will be important to sustainable crop development in future climate change scenarios, and dually for low-input practices that a majority of the world's farmers employ.

### Genetic bottlenecks may limit the possibilities for crop improvement under traditional breeding schemes

Recent large-scale studies underscore the importance of roots in global agriculture and ecology (Warren et al. 2014; West et al. 2014). A meta-analysis of Midwest U.S. crop production over the past few decades came to the striking conclusion that at the field scale, maize has become more sensitive to vapor pressure deficit-driven drought stress during modern breeding (Lobell et al. 2014). Similarly, despite the fact that maize agricultural hybrids have become increasingly more nitrogen use efficient (NUE), Gallais and Coque (2005) posited that modern cultivars may have lost root growth-related plasticity responses important for nitrogen uptake efficiency (NUpE) due to their typical selection under high-N inputs. This idea was supported by the findings of Chen et al. (2013), and has important implications for future development of precision and low-input agricultural schemes. A comparison

of green-revolution wheat cultivars with a global sampling of modern landraces found that these cultivars have substantially smaller root systems than landraces (Waines and Ehdaie 2007), which may limit stress resilience in low-input systems. Analysis of teosinte landraces (Burton et al. 2013) and the effect of mutations in the major *Teosinte Branched 1* (*Tb1*) domestication gene on maize root architecture came to similar conclusions (Gaudin et al. 2014). But most critically, it should be pointed out that most of the major genes/alleles conferring stress tolerance through root function have been found outside the predominant breeding germplasm. In the case of the *PUP1/Pstol1* low phosphorous tolerance trait, the gene was identified from the *aus*-type Kasalath landrace native to Northeast India and is apparently not present in the two dominant rice breeding varieties Nipponbare (*japonica*-type) and IR64 (*indica*-type) (Gamuyao et al. 2012; Schatz et al. 2014). For *Dro1*, a 1 bp mutation was identified in the IR64-derived breeding lines that conferred shallow roots and drought susceptibility, but not ancestral landraces or wild rice (Uga et al. 2013). Further, the salt-tolerance gene *TmHKT1;5-A* was found in a relative of durum wheat (Munns et al. 2012). Taken together, these lines of evidence point to our general ignorance of the effects of domestication-related bottlenecks and strong selective pressure for desirable aboveground traits (Wissuwa et al. 2009; Hufford et al. 2012) on root architecture and function. Roots that may be highly functional in current intensive agricultural settings may perform poorly or even maladaptively in low-input and future climate change scenarios. Thus, working solely within elite breeding germplasm is unlikely to effectively exploit the wealth of natural genetic variation at our disposal (Wissuwa et al. 2009).

### Identifying the genetic and mechanistic basis of root traits will improve our understanding and ability to manipulate ideotypes, tradeoffs and plasticity to accelerate agricultural improvement

C. M. Donald laid out the concept of ideotype breeding in 1968 (Donald 1968) as direct selection for certain model plant characters that contribute to yield (rather than yield itself, or elimination of yield defects). A central pillar of ideotype is that plant characters are selected to suit their prevailing environment. Not surprisingly, Donald had little to say about what traits a model root system would possess, but with knowledge accumulated 50 years since, the idea of “matching roots to their environment” has greatly advanced (White et al. 2013).

Indeed, there have been numerous recent successes in breeding specifically for root traits related to stress tolerance and resource use efficiency, particularly in common bean, soybean, maize, wheat and rice (Zheng et al. 2000; Zhu et al. 2005; Zhu et al. 2010; Beebe et al. 2006; Kirkegaard et al. 2007; Ao et al. 2010; Lynch 2011; Gregory et al. 2013; Miguel et al. 2013; Saengwilai et al. 2014a, 2014b; Wasson et al. 2014; Zhan and Lynch 2015). These and other works (Blum 2005; Rose et al. 2013) have also highlighted the importance of understanding the complex relationships between root form, function and their environmental interactions. In some environments, there are dual benefits for a single trait, such as steeper root angles in *Dro1-NIL* plants, which may confer both drought tolerance and decreased cadmium

uptake (Kitomi et al. 2015), or aluminum and low-P tolerance in sorghum (Leiser et al. 2014). Recent experiments in common bean clearly demonstrated that the synergism between multiple root traits can depend on placement and timing (Miguel et al. 2015). Specifically, root hairs can increase P uptake much more when co-localized with shallow roots, and vice-versa, since P is largely bound in superficial topsoil layers (Lynch and Wojciechowski 2015; Miguel et al. 2015). Thus, breeding for either of these two traits alone is far less efficient than together. Conversely, for a crop such as rice that is particularly susceptible to drought in many agricultural scenarios, the shallow-rooted trait shown to be beneficial for P uptake has a strong negative tradeoff (Ho et al. 2005). The concept of a dimorphic root system that has a percentage of shallow P-obtaining roots and a few deep roots for water uptake, would then become a valuable ideotype (Ho et al. 2005).

One criticism of the ideotype paradigm is that it is not comprehensive, that is it does not explicitly take into account the morphological, physiological and genetic relationships between traits of interest, and thus may be difficult to employ successfully in a breeding program (Rasmusson 1987; York et al. 2013). Clearly there are opportunities for positive and negative tradeoffs to plant productivity when intentionally breeding or modifying root traits, so how best to account and predict for them? Functional structural plant modeling is an accelerating framework on which to assemble a rapidly growing body of knowledge about plant growth characters and functional interactions with soil resources (Pierret et al. 2007). This approach models resource availability within the plant, and the consequences of growth “decisions” on the whole plant to generate a explicitly quantifiable hypothesis. Reinforcing the concept that more is not always better, a recent modeling study suggested an intermediate branching density for maize lateral roots was optimal for N and P uptakes (Postma et al. 2014), and similar studies suggest negative consequences for very dense crown roots (Saengwilai et al. 2014b). In these cases, the modeling predictions correlated well to the extent they were tested in the field. Yet models can only be as good as their assumptions, and the parameter values derived from them are unlikely to be hard targets for breeding until we know much more about relationships for whole-plant structure, function and environment.

Root growth plasticity *per se* is likely to be an ideotype of great value for efficient resource capture in a wide diversity of environments. Classic experiments demonstrated the ability of crop and wild plants to redistribute subterranean carbon resources to exploit soil nutrient patches (Drew and Saker 1975; Jackson and Caldwell 1989; Robinson et al. 1999; Watt and Evans 1999). However, while plasticity may be directly bred for, the highly heterogeneous and multivariate nature of field environments are likely to hinder the scope of application for any one plasticity trait. Therefore, to fully harness root plasticity for agriculture will require very detailed information about its mechanistic and genetic basis, as well as its environmental conditioning and tradeoffs. A body of work in *Arabidopsis* on agar plates has identified apparent plasticity genes that control the “tunability” of root architecture in response to nutrient availability (Zhang and Forde 1998; Svistoonoff et al. 2007; Ruffel et al. 2011; Gifford et al. 2013;

Gruber et al. 2013; Rosas et al. 2013; Araya et al. 2014; Yu et al. 2014), and a recent groundbreaking study identified molecular signals that integrate local root nutrient sensing with shoots and reciprocal control of root growth (Tabata et al. 2014). Continued development of *in vivo* nutrient sensors (Chen et al. 2010; Waadt et al. 2014), imaging systems that connect gene expression with morphological change (Busch et al. 2012; Grossmann et al. 2012; Rellan-Alvarez et al. 2015) and technologies to quantify carbon allocation dynamics in real time (Lee et al. 2013; Wang et al. 2014; Karve et al. 2015) can be expected to provide critical information about the molecular mechanisms of root-environment response and growth.

Breeding for root traits directly or indirectly will continue to drive crop improvement into the foreseeable future. However the discovery of genes underlying critical quantitative traits such as *Pstol1* in rice, and the *SbMate* family of aluminum tolerance transporters in sorghum (Magalhaes et al. 2007), have already spurred identification of their orthologs in other species for a more efficient crop improvement trajectory than could be achieved by trait selection-based breeding (Mickelbart et al. 2015). Novel phenotyping technologies for roots will eventually allow plant breeders to more effectively breed for specific root ideotypes tailored to their target environments, while at the same time allowing researchers to unravel the genetic mechanisms and variation controlling them.

## HOW CAN WE GENERATE A MORE COMPREHENSIVE VIEW OF ROOT SYSTEMS AND DEVELOP GOOD MODELS FOR WHOLE-PLANT FUNCTION?

Speaking the same language would help: The Root System Markup Language (RSML) is an XML-based format for describing topological and geometrical features and associated metadata of root phenotypes that constitutes a major step in this direction, as it provides a common translator for multiple root phenotyping software and allows for comparisons of traits measured from different sources (Lobet et al. 2015). OpenAlea is another flexible open source framework that uses visual programming for structural-functional plant modeling at scales from subcellular to whole plant (Pradal et al. 2008). Structure-function and other modeling frameworks such as deformable domains are a most powerful way to integrate multivariate data and generate testable hypotheses for the real world (Lynch et al. 1997; Pagès 2006; Dupuy et al. 2010; Leitner et al. 2010). Critically, such models incorporate time and multiple physical scales so that the aggregate shapes arising from the cumulative growth decisions of each meristem in the root system can be compared to empirically measured architectures. There is enormous potential to inform growth modeling parameters with experimental evidence from multiple sources, for example gene expression, hormone, nutrient and carbon flux, cell patterning and ontogenic information, as well as external features of the environment such as nutrient gradients and bulk water flow, soil physical parameters and microbial interactions (Pierret et al. 2007; Draye et al. 2010;

Dunbabin et al. 2011; Dunbabin et al. 2013). As increasingly well-parameterized models become more powerful, a key goal will be to link models of individual plant function to field and ecosystem scales (Norby and Jackson 2000; Kell 2012; Brown et al. 2014; Warren et al. 2014). However, modelling should be approached cautiously, and a consideration of scalar and dimensional interactions when combining multivariate traits is important (Niklas 1994; Vignaux and Scott 1999). The relationships among traits can change with physical and temporal scale (allometry), and this change is not always easily detected or well portrayed by regression models, especially when traits are measured in different dimensions and with different units. Dimension analysis, widely used in the physics and engineering fields, allows models to be checked for dimensional consistency by breaking derived traits (i.e., area, density, rate) down into basic units of measure (i.e., length, mass, time, amount) so that multivariate relationships can be compared at a fundamental level (Niklas 1994; Vignaux and Scott 1999).

Ultimately, there is no substitute for the power of genetics in relating genotype to phenotype, and we can use comparative genetic architecture to bridge data from different root phenotyping experiments. The most robust QTLs would be shared across multiple environments, different methods of trait quantification, or even across plant development, and could form a knowledge base from which to explore genes controlling more specific interactions of roots with environments. Focusing on a core mapping population or other sets of germplasm will make these direct comparisons more feasible. MetaQTL analysis has also been effectively used to combine different QTL experiments for higher confidence or resolution, and can provide clues to the contribution of roots to more complex traits such as yield and drought tolerance (Tuberosa et al. 2002b; Veyrieras et al. 2007; Courtois et al. 2009; Khowaja et al. 2009). Furthermore, since image-based analysis allows for the instant quantification of multiple aspects of root architecture from many samples, root phenotypes can now be defined with multivariate functions that exploit trait covariance information. Such multivariate phenotypes can be defined by principal component analysis, multivariate analysis of variance, or otherwise. Multivariate trait mapping has been used to identify new regions of the genome that were not identified from QTL analyses with *a priori* defined univariate traits (Anderson et al. 2011; Topp et al. 2013; Dixit et al. 2015; Marquez and Houle 2015). Finally, as we develop dynamic growth analysis tools that allow us to quantify how phenotypes change over time, we can begin to explore the genetic basis of infinite-dimensional, or function-valued traits that describe phenotypic characters as continuous equations rather than static values (Kirkpatrick and Heckman 1989; Stinchcombe and Kirkpatrick 2012; Kwak et al. 2014; Kwak et al. 2015; Bac-Molenaar et al. 2015). These methods have been developed in the ecology and evolution fields, but are now just starting to be applied to plant trait mapping in conjunction with advances in phenotyping throughput and automation. Considering phenotype in this way has transformative potential for our understanding of allometric growth, plant-environment interactions such as plasticity, and the dynamic relationships between root form and function.

## CONCLUSIONS

One of the most significant challenges of our time – to generate more yield with less input in rapidly changing environments – will rely on advances in our ability to explicitly manipulate plants, and thus to harness the genetic and phenotypic diversity of root architecture. While the obstacles to studying roots posed here and in other thoughtful works are well known, there is much evidence that our collective efforts are paying off with new and accelerating knowledge of root architecture at functional and genetic resolutions. Eventually a scenario can be envisioned where we can use short-term and highly localized predictions of weather and soil conditions to guide a targeted approach to rapid breeding or synthetic biology using ideotype or other modules for crop improvement (Brown et al. 2014). Data-driven model parameterization of plant function across scales is a daunting but critical aspect to this vision of the future (Hammer 2004). Ultimately, supporting terrestrial scale models with quality empirical data (Warren et al. 2014) will help us to effectively identify and address current and future leverage points in global food security (West et al. 2014).

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## REFERENCES

- Anderson JT, Lee CR, Mitchell-Olds T (2011) Life-history QTLs and natural selection on flowering time in *Boechnera stricta*, a perennial relative of *Arabidopsis*. **Evolution** 65: 771–787
- Andrade-Sanchez P, Gore MA, Heun JT, Thorp KR, Carmo-Silva AE, French AN, Salvucci ME, White JW (2014) Development and evaluation of a field-based high-throughput phenotyping platform. **Funct Plant Biol** 41: 68–79
- Ao J, Fu J, Tian J, Yan X, Liao H (2010) Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. **Funct Plant Biol** 37: 304–312
- Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, Tsuchiya YN, Sawa S, Fukuda H, Wirén von N, Takahashi H (2014) CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. **Proc Natl Acad Sci USA** 111: 2029–2034



- Bac-Molenaar JA, Vreugdenhil D, Granier C, Keurentjes JJB (2015) Genome-wide association mapping of growth dynamics detects time-specific and general quantitative trait loci. **J Exp Bot** 66: 5567–5580
- Baxter I, Dilkes BP (2012) Elemental profiles reflect plant adaptations to the environment. **Science** 336: 1661–1663
- Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE (2008) The leaf ionome as a multivariable system to detect a plant's physiological status. **Proc Natl Acad Sci USA** 105: 12081–12086
- Baxter IR, Ziegler G, Lahner B, Mickelbart MV, Foley R, Danku J, Armstrong P, Salt DE, Hoekenga OA (2014) Single-kernel ionic profiles are highly heritable indicators of genetic and environmental influences on elemental accumulation in maize grain (*Zea mays*). **PLoS ONE** 9: e87628
- Beebe SE, Rojas-Pierce M, Yan X, Blair MW, Pedraza F, Muñoz F, Tohme J, Lynch JP (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. **Crop Sci** 46: 413–423
- Bilder RM, Sabb FW, Cannon TD, London ED, Jentsch JD, Parker DS, Poldrack RA, Evans C, Freimer NB (2009) Phenomics: The systematic study of phenotypes on a genome-wide scale. **Neuroscience** 164: 30–42
- Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? **Aust J Agric Res** 56: 1159–1168
- Bohm W (1979) *Methods of Studying Root Systems*. Springer-Verlag. pp. 1–188
- Brekke B, Edwards J, Knapp A (2011a) Selection and adaptation to high plant density in the Iowa Stiff Stalk synthetic maize (*Zea mays* L.) population. **Crop Sci** 51: 1965–1972
- Brekke B, Edwards J, Knapp A (2011b) Selection and adaptation to high plant density in the Iowa Stiff Stalk synthetic maize (*Zea mays* L.) population: II. Plant morphology. **Crop Sci** 51: 2344–2351
- Brown TB, Cheng R, Sirault XRR, Rungrat T, Murray KD, Trtilek M, Furbank RT, Badger M, Pogson BJ, Borevitz JO (2014) TraitCapture: Genomic and environment modelling of plant phenomic data. **Curr Opin Plant Biol** 18: 73–79
- Bucksch A, BurrIDGE J, York LM, Das A, Nord E, Weitz JS, Lynch JP (2014) Image-based high-throughput field phenotyping of crop roots. **Plant Physiol** 166: 470–486
- Burton AL, Brown KM, Lynch JP (2013) Phenotypic diversity of root anatomical and architectural traits in species. **Crop Sci** 53: 1042–1055
- Busch W, Moore BT, Martsberger B, Mace DL, Twigg RW, Jung J, Pruteanu-Malinici I, Kennedy SJ, Fricke GK, Clark RL, Ohler U, Benfey PN (2012) A microfluidic device and computational platform for high-throughput live imaging of gene expression. **Nat Methods** 1–10
- Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB, Frommer WB (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. **Nature** 468: 527–532
- Chen X, Zhang J, Chen Y, Li Q, Chen F, Yuan L, Mi G (2013) Changes in root size and distribution in relation to nitrogen accumulation during maize breeding in China. **Plant Soil** 374: 121–130
- Chitwood DH, Topp CN (2015) Revealing plant cryptotypes: Defining meaningful phenotypes among infinite traits. **Curr Opin Plant Biol** 24: 54–60
- Chochois V, Vogel JP, Rebetzke GJ, Watt M (2015) Variation in adult plant phenotypes and partitioning among seed and stem-borne roots across brachypodium distachyon accessions to exploit in breeding cereals for well-watered and drought environments. **Plant Physiol** 168: 953–967
- Ciampitti IA, Vyn TJ (2012) Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. **Field Crop Res** 133: 48–67
- Clark R, MacCurdy R, Jung J, Shaff J, McCouch SR, Aneshansley D, Kochian L (2011) 3-Dimensional root phenotyping with a novel imaging and software platform. **Plant Physiol** 156: 455–465
- Colombi T, Kirchgessner N, Le Marié CA, York LM, Lynch JP, Hund A (2015) Next generation shovelomics: Set up a tent and REST. **Plant Soil** 388: 1–20
- Courtois B, Ahmadi N, Khowaja F, Price AH, Rami JF, Frouin J, Hamelin C, Ruiz M (2009) Rice root genetic architecture: Meta-analysis from a drought QTL database. **Rice** 2: 115–128
- Das A, Schneider H, BurrIDGE J, Ascanio AKM, Wojciechowski T, Topp CN, Lynch JP, Weitz JS, Bucksch A (2015) Digital imaging of root traits (DIRT): A high-throughput computing and collaboration platform for field-based root phenomics. **Plant Methods** 11: 51
- de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: Opportunities and constraints for genetic improvement of crops. **Trends Plant Sci** 12: 474–481
- Dixit S, Grondin A, Lee CR, Henry A, Olds TM, Kumar A (2015) Understanding rice adaptation to varying agro-ecosystems: Trait interactions and quantitative trait loci. **BMC Genet** 16: 1–15
- Donald CM (1968) The breeding of crop ideotypes. **Euphytica** 17: 385–403
- Downie HF, Adu MO, Schmidt S, Otten W, Dupuy LX, White PJ, Valentine TA (2015) Challenges and opportunities for quantifying roots and rhizosphere interactions through imaging and image analysis. **Plant Cell Environ** 38: 1213–1232
- Draye X, Kim Y, Lobet G, Javaux M (2010) Model-assisted integration of physiological and environmental constraints affecting the dynamic and spatial patterns of root water uptake from soils. **J Exp Bot** 61: 2145–2155
- Drew M, Saker L (1975) Nutrient supply and the growth of the seminal root system in barley. **J Exp Bot** 26: 79–90
- Dunbabin VM, Airey M, Diggle AJ, Renton M, Rengel Z, Armstrong R, Chen Y, Siddique K (2011) Simulating the interaction between plant roots, soil water and nutrient flows, and barriers and objects in soil using ROOTMAP. In: Chan F, Marinova D, Anderssen RS, eds. MODSIM2011, 19th International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand, December 2011. pp. 975–981 ISBN: 978-0-9872143-1-7. [www.mssanz.org.au/modsim2011/D10/wongsosaputro.pdf](http://www.mssanz.org.au/modsim2011/D10/wongsosaputro.pdf)
- Dunbabin VM, Postma JA, Schnepf A, Pagès L, Javaux M, Wu L, Leitner D, Chen YL, Rengel Z, Diggle AJ (2013) Modelling root-soil interactions using three-dimensional models of root growth, architecture and function. **Plant Soil** 372: 93–124
- Dupuy L, Gregory PJ, Bengough AG (2010) Root growth models: Towards a new generation of continuous approaches. **J Exp Bot** 61: 2131–2143
- Duvick D (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). **Adv Agron** 86: 83–145
- Fedoroff NV, Battisti DS, Beachy RN, Cooper PJM, Fischbach, Hodges CN, Knauft VC, Lobell D, Mazur BJ, Molden D, Reynolds MP, Ronald PC, Rosegrant MW, Sanchez PA, Vonshak A, Zhu JK (2010) Radically rethinking agriculture for the 21st Century. **Science** 327: 833–834

- Furbank RT, Tester M (2011) Phenomics-technologies to relieve the phenotyping bottleneck. **Trends Plant Sci** 16: 635–644
- Gallais A, Coque M (2005) Genetic variation and selection for nitrogen use efficiency in maize: A synthesis. **Maydica** 50: 531–547
- Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. **Nature** 488: 535–539
- Gaudin ACM, McClymont SA, Soliman SSM, Raizada MN (2014) The effect of altered dosage of a mutant allele of Teosinte branched 1 (tb1-ref) on the root system of modern maize. **BMC Genet** 15: 23
- Gifford ML, Banta JA, Katari MS, Hulsmans J, Chen L, Ristova D, Tranchina D, Purugganan MD, Coruzzi GM, Birnbaum KD (2013) Plasticity regulators modulate specific root traits in discrete nitrogen environments. **PLoS Genet** 9: e1003760
- Granier C, Vile D (2014) Phenotyping and beyond: Modelling the relationships between traits. **Curr Opin Plant Biol** 18: 96–102
- Gregory PJ, Atkinson CJ, Bengough AG, Else MA, Fernandez-Fernandez F, Harrison RJ, Schmidt S (2013) Contributions of roots and rootstocks to sustainable, intensified crop production. **J Exp Bot** 64: 1209–1222
- Griff TE, Novais J, Bohn M (2011) High-throughput phenotyping technology for maize roots. **Biosyst Eng** 110: 40–48
- Grossmann G, Guo WJ, Ehrhardt DW, Frommer WB, Sit RV, Quake SR, Meier M (2012) The RootChip: An integrated microfluidic chip for plant science. **Plant Cell** 23: 4234–4240
- Gruber BD, Giehl RFH, Friedel S, Wirén von N (2013) Plasticity of the *Arabidopsis* root system under nutrient deficiencies. **Plant Physiol** 163: 161–179
- Gutjahr C, Sawers RJH, Marti G, Andrés-Hernández L, Yang SY, Casieri L, Angliker H, Oakeley EJ, Wolfender JL, Abreu-Goodger C, Paszkowski U (2015) Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. **Proc Natl Acad Sci USA** 112: 6754–6759
- Hammer GL (2004) On systems thinking, systems biology, and the in silico plant. **Plant Physiol** 134: 909–911
- Hammer GL, Dong Z, Mclean G, Doherty A, Messina C, Schussler J, Zinselmeier C, Paszkiewicz S, Cooper M (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt? **Crop Sci** 49: 299
- Heeraman DA, Juma NG (1993) A comparison of minirhizotron, core and monolith methods for quantifying barley (*Hordeum vulgare* L.) and fababean (*Vicia faba* L.) root distribution. **Plant Soil** 148: 29–41
- Ho MD, Rosas JC, Brown KM, Lynch JP (2005) Root architectural tradeoffs for water and phosphorus acquisition. **Funct Plant Biol** 32: 737–748
- Houle D, Govindaraju DR, Omholt S (2010) Phenomics: The next challenge. **Nat Rev Genet** 11: 855–866
- Hufford MB, Xu X, van Heerwaarden J, Pyhäjärvi T, Chia JM, Cartwright RA, Elshire RJ, Glaubitz JC, Guill KE, Kaeppler SM, Lai J, Morrell PL, Shannon LM, Song C, Springer NM, Swanson-Wagner RA, Tiffin P, Wang J, Zhang G, Doebley J, McMullen MD, Ware D, Buckler ES, Yang S, Ross-Ibarra J (2012) Comparative population genomics of maize domestication and improvement. **Nat Genet** 44: 808–811
- Hufnagel B, de Sousa SM, Assis L, Guimaraes CT, Leiser W, Azevedo GC, Negri B, Larson BG, Shaff JE, Pastina MM, Barros BA, Weltzien E, Rattunde HF, Viana JH, Clark RT, Falcão A, Gazaffi R, Garcia AA, Schaffert RE, Kochian LV, Magalhaes JV (2014) Duplicate and conquer: Multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils. **Plant Physiol** 166: 659–677
- Iyer-Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Weitz JS, Benfey PN (2010) Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. **Plant Physiol** 152: 1148–1157
- Jackson R, Caldwell M (1989) The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. **Oecologia** 81: 149–153
- Jahnke S, Menzel MI, van Dusschoten D, Roeb GW, Bühler J, Minwuyet S, Blümler P, Temperton VM, Hombach T, Streun M, Beer S, Khodaverdi M, Ziemons K, Coenen HH, Schurr U (2009) Combined MRI-PET dissects dynamic changes in plant structures and functions. **Plant J** 59: 634–644
- Kaestner A, Schneebeli M, Graf F (2006) Visualizing three-dimensional root networks using computed tomography. **Geoderma** 136: 459–469
- Kanno S, Yamawaki M, Ishibashi H, Kobayashi NI, Hirose A, Tanoi K, Nussaume L, Nakanishi TM (2012) Development of real-time radioisotope imaging systems for plant nutrient uptake studies. **Philos Trans R Soc Lond B Biol Sci** 367: 1501–1508
- Karve AA, Alexoff D, Kim D, Schueller MJ, Ferrieri RA, Babst BA (2015) In vivo quantitative imaging of photoassimilate transport dynamics and allocation in large plants using a commercial positron emission tomography (PET) scanner. **BMC Plant Biol** 15: 1–11
- Kell DB (2012) Large-scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: Why and how. **Philos Trans R Soc Lond B Biol Sci** 367: 1589–1597
- Khowaja FS, Norton GJ, Courtois B, Price AH (2009) Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. **BMC Genomics** 10: 276
- Kirkegaard JA, Lilley JM, Howe GN, Graham JM (2007) Impact of subsoil water use on wheat yield. **Aust J Agric Res** 58: 303–315
- Kirkpatrick M, Heckman N (1989) A quantitative genetic model for growth, shape, reaction norms, and other infinite-dimensional characters. **J Math Biol** 27: 429–450
- Kiser MR, Reid CD, Crowell AS, Phillips RP, Howell CR (2008) Exploring the transport of plant metabolites using positron emitting radiotracers. **HFSP J** 2: 189–204
- Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S, Uga Y (2015) QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. **Rice** 8: 16. doi: 10.1186/s12284-015-0049-2
- Kuijken RCP, van Eeuwijk FA, Marcelis LFM, Bouwmeester HJ (2015) Root phenotyping: From component trait in the lab to breeding. **J Exp Bot** 66: 5389–5401
- Kutschera L (1960) Wurzelatlas mitteleuropäischer Ackerunkräuter und Kulturpflanzen. DLG Verlag, Frankfurt-am-Main
- Kücker M, Schmid H, Spiess A (1995) A comparison of four methods for measuring roots of field crops in three contrasting soils. **Plant Soil** 172: 63–71
- Kwak IY, Moore CR, Spalding EP, Broman KW (2015) Mapping quantitative trait loci underlying function-valued traits using functional principal component analysis and multi-trait mapping. **G3 (Bethesda)** 6: 79–86
- Kwak IY, Moore CR, Spalding EP, Broman KW (2014) A simple regression-based method to map quantitative trait loci underlying function-valued phenotypes. **Genetics** 197: 1409–1416
- Lee S, Kross B, McKisson J, McKisson JE, Weisenberger AG, Xi W, Zorn C, Bonito G, Howell CR, Reid CD, Crowell A, Cumberbatch LC, Topp

- C, Smith MF (2013) Imaging corn plants with PhytoPET, a modular PET system for plant biology. *2013 IEEE Nuclear Science Symposium and Medical Imaging Conference (2013 NSS/MIC)*. IEEE. pp. 1–3
- Leiser WL, Rattunde HFW, Weltzien E, Cisse N, Abdou M, Diallo A, Tourè AO, Magalhaes JV, Haussmann BIG (2014) Two in one sweep: Aluminum tolerance and grain yield in P-limited soils are associated to the same genomic region in West African sorghum. *BMC Plant Biol* 14: 206. doi: 10.1186/s12870-014-0206-6
- Leitner D, Klepsch S, Bodner G, Schnepf A (2010) A dynamic root system growth model based on L-Systems. *Plant Soil* 332: 177–192
- Liu F, Wollstein A, Hysi PG, Ankra-Badu GA, Spector TD, Park D, Zhu G, Larsson M, Duffy DL, Montgomery GW, Mackey DA, Walsh S, Lao O, Hofman A, Rivadeneira F, Vingerling JR, Uitterlinden AG, Martin NG, Hammond CJ, Kayser M (2010) Digital quantification of human eye color highlights genetic association of three new loci. *PLoS Genet* 6: e1000934
- Lobell DB, Roberts MJ, Schlenker W, Braun N, Little BB, Rejesus RM, Hammer GL (2014) Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science* 344: 516–519
- Lobet G, Draye X, Perilleux C (2013) An online database for plant image analysis software tools. *Plant Methods* 9: 38
- Lobet G, Pound MP, Diener J, Pradal C, Draye X, Godin C, Javaux M, Leitner D, Meunier F, Nacry P, Pridmore TP, Schnepf A (2015) Root system markup language: Toward a unified root architecture description language. *Plant Physiol* 167: 617–627
- Lucas CJ, Zhao H, Schneerman M, Moose SP (2013) Genomic changes in response to 110 cycles of selection for seed protein and oil concentration in maize. In: Becraft PW, ed. *Seed Genomics*. Wiley-Blackwell, Oxford, UK. doi: 10.1002/978111852524.ch12
- Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiol* 156: 1041–1049
- Lynch JP, Wojciechowski T (2015) Opportunities and challenges in the subsoil: Pathways to deeper rooted crops. *J Exp Bot* 66: 2199–2210
- Lynch JP, Nielsen KL, Davis RD, Jablókow AG (1997) SimRoot: Modelling and visualization of root systems. *Plant Soil* 188: 139–151
- Maeght JL, Rewald B, Pierret A (2013) How to study deep roots-and why it matters. *Front Plant Sci* 4: 299. doi: 10.3389/fpls.2013.00299
- Magalhaes JV, Liu J, Guimarães CT, Lana UGP, Alves VMC, Wang YH, Schaffert RE, Hoekenga OA, Piñeros MA, Shaff JE, Klein PE, Carneiro NP, Coelho CM, Trick HN, Kochian LV (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat Genet* 39: 1156–1161
- Marquez EJ, Houle D (2015) Dimensionality and the statistical power of multivariate genome-wide association studies. *BioRxiv* 1–38
- McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010) Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: Energy dispersive X-ray microanalysis (CEDX) applications. *Funct Plant Biol* 37: 1011–1030
- Meijón M, Satbhai SB, Tsuchimatsu T, Busch W (2014) Genome-wide association study using cellular traits identifies a new regulator of root development in *Arabidopsis*. *Nat Genet* 46: 77–81
- Meister R, Rajani MS, Ruzicka D, Schachtman DP (2014) Challenges of modifying root traits in crops for agriculture. *Trends Plant Sci* 19: 779–788
- Metzner R, Eggert A, van Dusschoten D, Pflugfelder D, Gerth S, Schurr U, Uhlmann N, Jahnke S (2015) Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: Potential and challenges for root trait quantification. *Plant Methods* 11: 17
- Mickelbart MV, Hasegawa PM, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nat Publishing Group* 16(4): 1–15
- Miguel MA, Postma JA, Lynch JP (2015) Phene synergism between root hair length and basal root growth angle for phosphorus acquisition. *Plant Physiol* 167: 1430–1439
- Miguel MA, Widrig A, Vieira RF, Brown KM, Lynch JP (2013) Basal root whorl number: A modulator of phosphorus acquisition in common bean (*Phaseolus vulgaris*). *Ann Bot* 112: 973–982
- Mooney SJ, Pridmore TP, Helliwell J, Bennett MJ (2011) Developing X-ray Computed Tomography to non-invasively image 3-D root systems architecture in soil. *Plant Soil* 352(1–2): 1–22
- Moore CR, Johnson LS, Kwak I-Y, Livny M, Broman KW, Spalding EP (2013) High-throughput computer vision introduces the time axis to a quantitative trait map of a plant growth response. *Genetics* 195: 1077–1086
- Moose SP, Dudley JW, Rocheford TR (2004) Maize selection passes the century mark: A unique resource for 21st century genomics. *Trends Plant Sci* 9: 358–364
- Moradi AB, Conesa HM, Robinson B, Lehmann E, Kuehne G, Kaestner A, Oswald S, Schulin R (2008) Neutron radiography as a tool for revealing root development in soil: Capabilities and limitations. *Plant Soil* 318: 243–255
- Moran CJ, Pierret A, Stevenson AW (2000) X-ray absorption and phase contrast imaging to study the interplay between plant roots and soil structure. *Plant Soil* 223: 101–117
- Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD, Tester M, Plett D, Gilliam M (2012) Wheat grain yield on saline soils is improved by an ancestral Na<sup>+</sup> transporter gene. *Nat Biotech* 30: 360
- Nagel KA, Putz A, Gilmer F, Heinz K, Fischbach A, Pfeifer J, Faget M, Blossfeld S, Ernst M, Dimaki C, Kastenholz B, Kleinert A-K, Galinski A, Schar H, Fiorani F, Schurr U (2012) GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. *Funct Plant Biol* 39: 891–904
- Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere—The plant science toolbox. *Plant Soil* 321: 431–456
- Niklas KJ (1994) *Plant Allometry: The Scaling of Form and Process*. University of Chicago Press, Chicago
- Norby RJ, Jackson RB (2000) Root dynamics and global change: Seeking an ecosystem perspective. *New Phytol* 147: 3–12
- Pagès L (2006) Chapter 22: Modeling root system architecture. *Plant Roots the Hidden Half*, Third Edition. CRC Press, Taylor & Francis Group, Boca Raton, FL. pp. 1–24
- Pankiewicz VCS, do Amaral FP, Santos KFDN, Agtuca B, Xu Y, Schueller MJ, Arisi ACM, Steffens MBR, de Souza EM, Pedrosa FO, Stacey G, Ferrieri RA (2015) Robust biological nitrogen fixation in a model grass-bacterial association. *Plant J* 81: 907–919
- Pfeifer J, Kirchgessner N, Colombi T, Walter A (2015) Rapid phenotyping of crop root systems in undisturbed field soils using X-ray computed tomography. *Plant Methods* 11: 1–8
- Pierret A, Moran CJ (1996) Quantification of orientation of pore patterns in X-ray images of deformed clay. *Microsc Microanal Microstruct* 7: 421–431
- Pierret A, Doussan C, Capowiez Y, Bastardie F, Pagès L (2007) Root functional architecture: A framework for modeling the interplay between roots and soil. *Vadose Zone J* 6: 269

- Pierret A, Moran CJ, Doussan C (2005) Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. **New Phytol** 166: 967–980
- Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. **Plant Physiol** 166: 590–602
- Pradal C, Dufour-Kowalski S, Boudon F, Fournier C, Godin C (2008) OpenAlea: A visual programming and component-based software platform for plant modelling. **Funct Plant Biol** 35: 751
- Rasmusson DC (1987) An evaluation of ideotype breeding. **Crop Sci** 27: 1140–1146
- Rellán-Alvarez R, Lobet G, Lindner H, Pradier P-L, Sebastian J, Yee M-C, Geng Y, Trontin C, LaRue T, Schrager-Lavelle A, Haney CH, Nieu R, Maloof J, Vogel JP, Dinneny JR (2015) GLO-Roots: An imaging platform enabling multidimensional characterization of soil-grown root systems. **Elife** 4: 1–26
- Rengel D, Arribat S, Maury P, Martin-Magniette M-L, Hourlier T, Laporte M, Varès D, Carrère S, Grieu P, Balzergue S, Gouzy J, Vincourt P, Langlade NB (2012) A gene-phenotype network based on genetic variability for drought responses reveals key physiological processes in controlled and natural environments. **PLoS ONE** 7: e45249
- Robinson D, Hodge A, Griffiths B, Fitter A (1999) Plant root proliferation in nitrogen-rich patches confers competitive advantage. **Proc Royal Soc London Series B Biol Sci** 266: 431
- Rosas U, Cibrian-Jaramillo A, Ristova D, Banta JA, Gifford ML, Fan AH, Zhou RW, Kim GJ, Krouk G, Birnbaum KD, Purugganan MD, Coruzzi GM (2013) Integration of responses within and across *Arabidopsis* natural accessions uncovers loci controlling root systems architecture. **Proc Natl Acad Sci USA** 110: 15133–15138
- Rose TJ, Impa SM, Rose MT, Pariasca-Tanaka J, Mori A, Heuer S, Johnson-Beebout SE, Wissuwa M (2013) Enhancing phosphorus and zinc acquisition efficiency in rice: A critical review of root traits and their potential utility in rice breeding. **Ann Bot** 112: 331–345
- Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM (2011) Nitrogen economics of root foraging: Transitive closure of the nitrate–cytokinin relay and distinct systemic signaling for N supply vs. demand. **Proc Natl Acad Sci USA** 108: 18524–18529
- Saengwilai P, Nord EA, Chimungu JG, Brown KM, Lynch JP (2014a) Root cortical aerenchyma enhances nitrogen acquisition from low-nitrogen soils in maize. **Plant Physiol** 166: 726–735
- Saengwilai P, Tian X, Lynch JP (2014b) Low crown root number enhances nitrogen acquisition from low-nitrogen soils in maize. **Plant Physiol** 166: 581–589
- Schatz MC, Maron LG, Stein JC, Wences AH, Gurtowski J, Biggers E, Lee H, Kramer M, Antoniou E, Ghiban E, Wright MH, Chia J-M, Ware D, McCouch SR, McCombie WR (2014) Whole genome *de novo* assemblies of three divergent strains of rice, *Oryza sativa*, document novel gene space of aus and indica. **Genome Biol** 15: 506
- Schulz H, Postma JA, van Dusschoten D, Scharf H, Behnke S (2012) 3D reconstruction of plant roots from MRI images. *Proceedings of the International Conference on Computer Vision Theory and Applications VISAPP*. Springer-Verlag, Berlin, Heidelberg. pp. 1–9
- Shrestha R, Al-Shugeairy Z, Al-Ogaidi F, Munasinghe M, Radermacher M, Vandenhort J, Price AH (2014) Comparing simple root phenotyping methods on a core set of rice genotypes. **Plant Biol J** 16: 632–642
- Slovak R, Göschl C, Su X, Shimotani K, Shiina T, Busch W (2014) A scalable open-source pipeline for large-scale root phenotyping of *Arabidopsis*. **Plant Cell** 26: 2390–2403
- Spalding EP, Miller ND (2013) Image analysis is driving a renaissance in growth measurement. **Curr Opin Plant Biol** 16: 100–104
- Sperry TM (1935) Root systems in Illinois prairie. **Ecology** 16: 178
- Stinchcombe JR, Kirkpatrick M (2012) Genetics and evolution of function-valued traits: Understanding environmentally responsive phenotypes. **Trends Ecol Evol** 27: 637–647
- Subramanian R, Spalding EP, Ferrier NJ (2012) A high throughput robot system for machine vision based plant phenotype studies. **Mach Vision Appl** 24: 619–636
- Suwa R, Fujimaki S, Suzui N, Kawachi N, Ishii S, Sakamoto K, Nguyen NT, Saneoka H, Mohapatra PK, Moghaieb RE, Matsuhashi S, Fujita K (2008) Use of positron-emitting tracer imaging system for measuring the effect of salinity on temporal and spatial distribution of <sup>11</sup>C tracer and coupling between source and sink organs. **Plant Sci** 175: 210–216
- Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T (2007) Root tip contact with low-phosphate media reprograms plant root architecture. **Nat Genet** 39: 792–796
- Symonova O, Topp CN, Edelsbrunner H (2015) Dynamic roots: A software platform for the reconstruction and analysis of growing plant roots. **PLoS ONE** 10: e0127657
- Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y (2014) Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. **Science** 346: 343–346
- Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. **Nature** 418: 671–677
- Topp CN, Iyer-Pascuzzi AS, Anderson JT, Lee CR, Zurek PR, Symonova O, Zheng Y, Bucksch A, Mileyko Y, Galkovskiy T, Moore BT, Harer J, Edelsbrunner H, Mitchell-Olds T, Weitz JS, Benfey PN (2013) 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. **Proc Natl Acad Sci USA** 110: E1695–1704
- Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2010) Shovelomics: High throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. **Plant Soil** 341: 75–87
- Tsukamoto T, Nakanishi H, Uchida H, Watanabe S, Matsuhashi S, Mori S, Nishizawa NK (2009) <sup>52</sup>Fe Translocation in Barley as Monitored by a Positron-Emitting Tracer Imaging System (PETIS): Evidence for the direct translocation of Fe from roots to young leaves via phloem. **Plant Cell Physiol** 50: 48–57
- Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002a) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. **Plant Mol Biol** 48: 697–712
- Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S (2002b) Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. **Ann Bot** 89: 941–963
- Uga Y, Okuno K, Yano M (2011) *Dro1*, a major QTL involved in deep rooting of rice under upland field conditions. **J Exp Bot** 62: 2485–2494
- Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. **Nat Genet** 45: 1097–1102

- Uribelarrea M, Moose S, Below F (2007) Divergent selection for grain protein affects nitrogen use in maize hybrids. **Field Crop Res** 100: 82–90
- USDA, NSF (2011) *Phenomics: Genotype to Phenotype: A Report of the Phenomics Workshop* sponsored by the USDA and NSF. 1–42
- Veyrieras J-B, Goffinet B, Charcosset A (2007) MetaQTL: A package of new computational methods for the meta-analysis of QTL mapping experiments. **BMC Bioinformatics** 8: 49
- Vignaux VA, Scott JL (1999) Theory & methods: Simplifying regression models using dimensional analysis. **Aust New Zealand J Statistics** 41: 31–41
- Waadt R, Hitomi K, Nishimura N, Hitomi C, Adams SR, Getzoff ED, Schroeder JI (2014) FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in *Arabidopsis*. **Elife** 3: e01739
- Waines JG, Ehdaie B (2007) Domestication and crop physiology: Roots of green-revolution wheat. **Ann Bot** 100: 991–998
- Wang Q, Mathews AJ, Li K, Wen J, Komarov S, O'Sullivan JA, Tai YC (2014) A dedicated high-resolution PET imager for plant sciences. **Phys Med Biol** 59: 5613–5629
- Warren JM, Bilheux H, Kang M, Voisin S, Cheng CL, Horita J, Perfect E (2013) Neutron imaging reveals internal plant water dynamics. **Plant Soil** 366: 683–693
- Warren JM, Hanson PJ, Iversen CM, Kumar J, Walker AP, Wullschlegel SD (2014) Root structural and functional dynamics in terrestrial biosphere models – evaluation and recommendations. **New Phytol** 205: 59–78
- Wasson AP, Rebetzke GJ, Kirkegaard JA, Christopher J, Richards RA, Watt M (2014) Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. **J Exp Bot** 65: 6231–6249
- Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. **J Exp Bot** 63: 3485–3498
- Watt M, Evans J (1999) Proteoid roots. Physiology and development. **Plant Physiol** 121: 317–324
- Weaver JE (1926) *Root Development of Field Crops*. McGraw-Hill, New York
- Weaver JE, Voigt JW (1950) Monolith method of root-sampling in studies on succession and degeneration. **Bot Gazette** 111: 286–299
- Weaver JE, Jean FC, Crist JW (1922) *Development and Activities of Roots of Crop Plants: A Study in Crop Ecology*. Carnegie Institution of Washington
- West PC, Gerber JS, Engstrom PM, Mueller ND, Brauman KA, Carlson KM, Cassidy ES, Johnston M, MacDonald GK, Ray DK, Siebert S (2014) Leverage points for improving global food security and the environment. **Science** 345: 325–328
- White PJ, George TS, Gregory PJ, Bengough AG, Hallett PD, McKenzie BM (2013) Matching roots to their environment. **Annals of Bot** 112: 207–222
- Wishart J, George TS, Brown LK, Ramsay G, Bradshaw JE, White PJ, Gregory PJ (2012) Measuring variation in potato roots in both field and glasshouse: The search for useful yield predictors and a simple screen for root traits. **Plant Soil** 368: 231–249
- Wissuwa M, Mazzola M, Picard C (2009) Novel approaches in plant breeding for rhizosphere-related traits. **Plant Soil** 321: 409–430
- Wissuwa M, Wegner J, Ae N, Yano M (2002) Substitution mapping of Pup1: A major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. **Theor Appl Genet** 105: 890–897
- York LM, Galindo-Castaneda T, Schussler JR, Lynch JP (2015) Evolution of US maize (*Zea mays* L.) root architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress. **J Exp Bot** 66: 2347–2358
- York LM, Nord EA, Lynch JP (2013) Integration of root phenes for soil resource acquisition. **Front Plant Sci** 4: 355
- Yu P, Hochholdinger F, Li C (2015) Root-type-specific plasticity in response to localized high nitrate supply in maize (*Zea mays*). **Ann Bot** 116: 751–762
- Yu P, White PJ, Hochholdinger F, Li C (2014) Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. **Planta** 240: 667–678
- Zarebanadkouki M, Kim YX, Carminati A (2013) Where do roots take up water? Neutron radiography of water flow into the roots of transpiring plants growing in soil. **New Phytol** 199: 1034–1044
- Zarebanadkouki M, Kroener E, Kaestner A, Carminati A (2014) Visualization of root water uptake: Quantification of deuterated water transport in roots using neutron radiography and numerical modeling. **Plant Physiol** 166: 487–499
- Zhan A, Lynch JP (2015) Reduced frequency of lateral root branching improves N capture from low-N soils in maize. **J Exp Bot** 66: 2055–2065
- Zhang H, Forde BG (1998) An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. **Science** 279: 407–409
- Zheng HG, Babu RC, Pathan MS, Ali L, Huang N, Courtois B, Nguyen HT (2000) Quantitative trait loci for root-penetration ability and root thickness in rice: Comparison of genetic backgrounds. **Genome** 43: 53–61
- Zhu J, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). **Plant Cell Environ** 33: 740–749
- Zhu J, Kaeppeler SM, Lynch JP (2005) Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). **Funct Plant Biol** 32: 749–762
- Zurek PR, Topp CN, Benfey PN (2015) Quantitative trait locus mapping reveals regions of the maize genome controlling root system architecture. **Plant Physiol** 167: 1487–1496