

Review

Crop Improvement from Phenotyping Roots: Highlights Reveal Expanding Opportunities

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Root systems determine the water and nutrients for photosynthesis and harvested products, underpinning agricultural productivity. We highlight 11 programs that integrated root traits into germplasm for breeding, relying on phenotyping. Progress was successful but slow. Today's phenotyping technologies will speed up root trait improvement. They combine multiple new alleles in germplasm for target environments, in parallel. Roots and shoots are detected simultaneously and nondestructively, seed to seed measures are automated, and field and laboratory technologies are increasingly linked. Available simulation models can aid all phenotyping decisions. This century will see a shift from single root traits to rhizosphere selections that can be managed dynamically on farms and a shift to phenotype-based improvement to accommodate the dynamic complexity of whole crop systems.

Root System Traits Have Ongoing Value for Global Productivity

Root system **traits** (see [Glossary](#)) have long been a key target by researchers and breeders for crop improvement [1,2]. **Root system architecture** supplies water and nutrients for photosynthesis and growth, stabilizes the plant, and prevents soil toxic elements and pathogens from entering leaves and reproductive organs. Roots also host soil microorganisms that can contribute to plant growth and resource efficiency and modulate the supply of resources and signals to shoots, influencing partitioning to organs, including flowers, seed, and fruit. Despite the challenges that plant roots present for measurement, root traits have been incorporated into crops using phenotyping. The observation of roots using **phenotyping** is central to the discovery of root traits beneficial to crops, their incorporation into new cultivars using **prebreeding** [3,4], and to their management using precision agriculture.

The first section of the review highlights 11 programs that selected root traits that enhanced crop plant **productivity** using phenotyping. The highlights are used in the second section to show how emerging phenotyping and modeling methods can speed up future selection programs. Contemporary, automated, multisensor, and nondestructive technologies select whole-plant, dynamic and functional **phenotypes** into plants that can be grown to seed, speeding up advancement of beneficial alleles. Further gains are expected from integrating field phenotyping within selection programs using technologies to measure roots in soil (see companion Technology of the Month paper, was Wasson *et al.* [47]). We present five mathematical models to integrate whole-plant phenotyping programs, targeted at developing germplasm for farming systems to optimize trait expression and value to breeders farmers. In the third and final section of the review, we cover opportunities to discover new below-ground traits by phenotyping the **rhizosphere**. Rhizosphere phenotyping will lead to a forward development of genotypes matched to soil inputs and interventions for a beneficial, holistic root-soil zone. Rhizosphere phenotyping opens exciting challenges for research, including combinatorial factor experiments, repeatability across research programs, use of machine learning to extract new traits from images, and a new conceptual framework that addresses the limitations of single trait solutions to crop productivity by taking a **systems of systems** approach.

Highlights in Root Phenotyping

The 11 programs highlighted in [Figure 1](#) (Key Figure) exemplify root traits incorporated into new genotypes by phenotyping to increase crop productivity. The examples cover the two main plant types and root system developments: monocotyledons (two major cereal crops, wheat and rice) with seed and stem-borne roots with no cambial thickening, and dicotyledons (two major legume crops, bean

Highlights

Root and rhizosphere traits have been selected and incorporated into germplasm since the 1970s, proving the value of roots and phenotyping in prebreeding programs.

Past examples show how today's noninvasive phenotyping technologies that measure roots, shoots, and seeds, can be strategically combined to speed up germplasm enhancement.

Models are available to test and incorporate root phenotypes at different stages of selection programs.

The root-soil rhizosphere can be phenotyped noninvasively in soils, revealing new combinatorial traits relevant to the reality of farming systems and to select for crop improvement.

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Key Figure

Examples of Root Traits Selected by Phenotyping and Incorporated into New Germplasm for Breeders

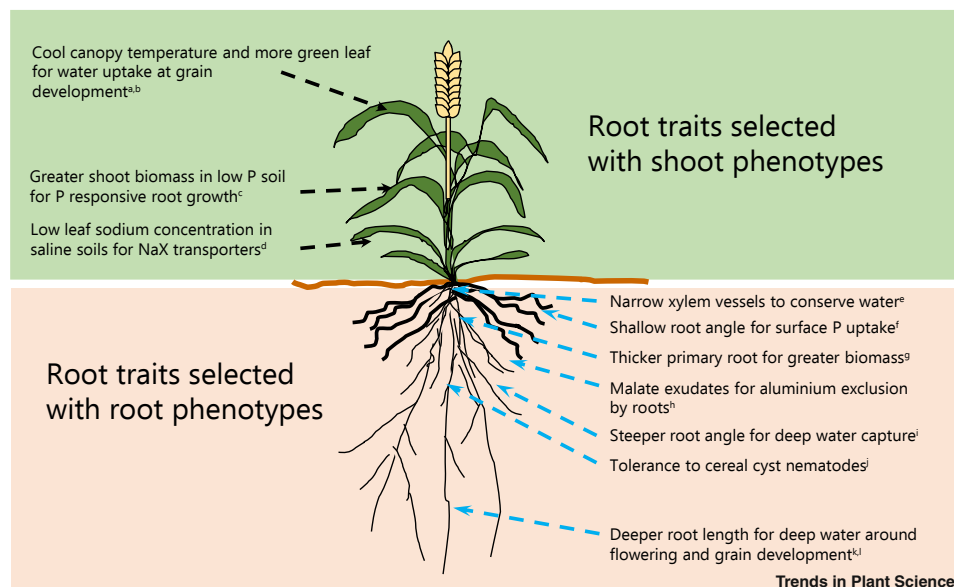


Figure 1. Traits are selected directly with root phenotyping and/or indirectly with shoot phenotyping. Examples are restricted to abiotic soil limitations. Phenotypes for deep water capture have been: deeper and more extensive root growth selected directly in rhizoboxes^k [7] and with field coring^l [9] and indirectly with canopy temperature and greenness^{a,b} [18,19,21]; and steeper roots (wide angle to soil surface) selected directly in baskets in pots of soilⁱ [8]. To conserve water for grain development, a narrow xylem anatomy was directly selected using a macroscope and hand sectioning^e [10]. Greater yield in drought-prone areas was increased by selecting for thicker tap roots along with optimal leaf width^g [11]. Greater P use with shallower roots was selected directly on paper pouches^f [12] and indirectly with shoot biomass on low P soil^c [13]. Transporters for malate at root tips were identified directly in a solution culture screen, conferring tolerance to acidic and high Al soils^h [14], and transporters within roots and the leaf sheath were identified with a shoot screen in controlled conditions^d [15]. See text for additional details.

and white clover) with tap root systems and secondary, cambial thickening (see Figure 1 of [5] for features of two root system types). Functional root phenotypes [6] were selected to confer: increased water acquisition [7–9], water use efficiency (WUE) [10], drought tolerance [11], access to phosphorus [12,13], and tolerance to high soil Al [14], Na [15], and cereal cyst nematodes [16], representing the dominant targets for which breeders want to improve roots [1]. The research in Figure 1 all reached, as a minimum, parental germplasm with the desired phenotype to use in a breeding program to generate a cultivar for farmers. The phenotyping techniques were deployed at different parts of the programs: (i) for parental identification only (e.g., [7] and [9]) followed by progeny selection using yield or canopy measures; (ii) for parental and progeny selections [10,11]; and (iii) for marker development (see examples in [17]). This indicates that even to date, direct and indirect phenotyping for roots has been incorporated into all aspects of germplasm enhancement.

Some selections commenced with a broad genetic diversity in germplasm from which to find the contrast in phenotype (e.g., [10]), while others were biased towards germplasm from the target environments where the resource of interest was limited [7,9,12]. Shoots or roots were the starting point for phenotyping. For example, Munns and team [15] commenced in a controlled

Glossary

Deep phenotyping: the precise and comprehensive analysis of phenotypic parts, whereby the individual components of the phenotype are observed and described (from precision medicine [117]); many layers (spatial or temporal) of information about the phenotype (from mathematical neural networks).

Holobiont: the host plant and its associated microbiota, such that holobiont is the unit of selection ([116] and references therein).

Model: a representation of a system that allows for investigation of the properties of the system and, in some cases, prediction of future outcomes (<http://www.investorwords.com/5662/model.html#ixzz5GgbCAKuv>).

Phenotype: the entirety of an organism's structural and functional expressed features, or one or a subset of an organism's structural and functional features. Depending on the discipline, phenotype can be derived from protein, metabolite, and gene composition and their state.

Phenotyping: the activity of qualifying and quantifying observable features of organisms.

Prebreeding: all activities designed to identify desirable characteristics or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers [79].

Productivity: output per unit input; for example, in agriculture, yield per rainfall.

Rhizosphere: rhizosphere is the term, attributed to Lorenz Hiltner, used in 1904 for the 'soil influenced by roots in terms of the microorganisms' [118]. Here, we propose to explicitly include the root within the definition of the rhizosphere.

Rhizosphere phenotype: root and root-influenced soil describing 'the manifestation of a plant's genetics' in the soil [94].

Root system architecture: the spatial configuration of the root system, that is, the explicit geometric deployment of root axes [119] of all roots of a plant, determined by elongation,

environment system and selected for low salt accumulation in leaves, identifying markers for sodium exclusion from root and leaf sheath tissues. Wissuwa and colleagues [13], commenced in the field by selecting shoot performance after tillering in low P conditions using destructive plant biomass and nutrient contents, progressing to identification of a root quantitative trait locus (QTL) associated with nodal root initiation. Reynolds and colleagues selected for canopy temperature at various time points within wheat crop growth in fields, as an indication of root distribution [18,19]. In the case of Wasson, Richards, and colleagues, deep rooted lines were identified first by direct coring in the field [20] and then canopy greenness was used in the field on progeny lines to speed up development of adapted germplasm carrying deep roots conferring water uptake [21]. The program of Caradus and Woodfield was particularly forward looking, as they successfully increased tap root thickness while maintaining an optimal leaf area by co-selecting root and leaf thickness with calipers in the field over several breeding cycles [11]. Molecular markers for genes underlying the root phenotypes were identified in five of the examples [8,13–16], but critically, the lack of a genome region for the trait did not stop the development of new germplasm for breeders in the other cases. These programs took 5 to more than 10 years from trait idea to new germplasm.

Opportunities to Speed Up Root Improvements with New Phenotyping Technologies and Simulation Models

What can we learn from these highlights to speed up future root improvement programs? The activities in Figure 1 have similarities. All commenced with phenotyping for a single, preconceived, heritable root trait. Controlled environment and then field activities were carried out in a series, from trait identification, to root and shoot phenotyping, to phenotype introgression into elite germplasm. The aim was to incorporate the root phenotype with beneficial traits associated with yield, quality, and disease resistances at the end of the program. Here, we highlight how today's phenotyping technologies can be deployed in new parallel and strategic orders of operation, rather than a series, to speed up future selection programs for roots.

Whole-Plant Phenotyping: Simultaneous Measurement of Root and Shoot Phenotypes

Automated phenotyping platforms quantify roots and shoots simultaneously (Figure 2A) [22,23]. Shoot phenotypes are not always unique to the root phenotype required for a particular soil condition (illustrated in Figure 2 in [24]). Phenotypes in roots and shoots are expressed differently depending on soil conditions (e.g., nitrogen [25], water [26,27]), strength [27,28], or shoot resources (e.g., light [27,29]). Phenotyping platforms identify genetic variation in partitioning among shoot structure (branching and tillering) [30] and roots (seed and stem borne roots) [31,32], and how these change with soil moisture [33] or gradients in soil conditions [28]. By selecting shoot and root traits simultaneously and directly, it is possible to make genetic gain within the whole plant quicker than selecting on shoot or root traits alone. Platforms such as that exemplified in Figure 2A scan 60 root systems per hour (automated), and five root systems per person hour is required after scanning [22]. This throughput is suitable to discover new traits, identify parents for breeding, QTL analyses, and proof of concept studies. A new platform with the capability for hundreds of rhizotrons will have the capacity to do progeny and elite germplasm development (F. Fiorani, personal communication).

Functional and Dynamic Phenotypes for Capture of Soil Resources

Breeders target functional traits, such as WUE and nitrogen use efficiency (NUE). Phenotyping platforms incorporate water and nutrient measurements in the soil and the plant to quantify genotypes that vary in WUE [34], NUE [25], and salinity tolerance [35]. These platforms move plants, using automated robotics, to imaging and weighing stations and sensors, and throughput is high enough to identify genetic variation for further prebreeding.

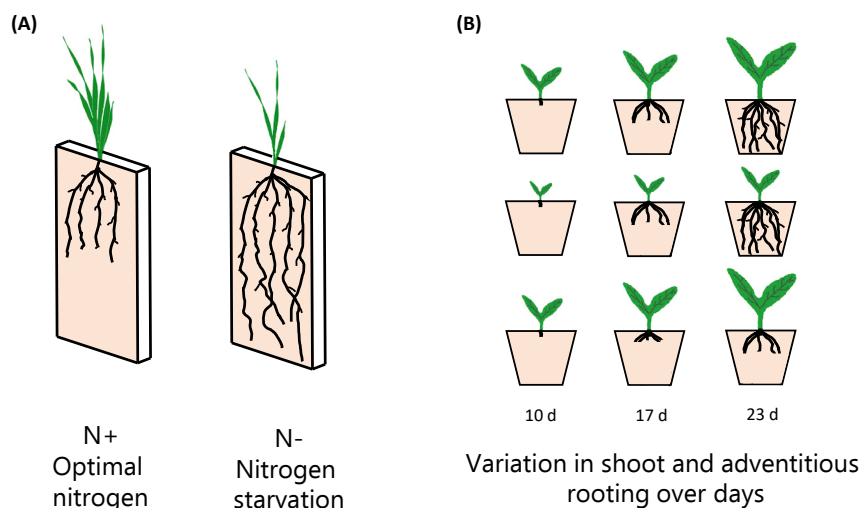
Root and shoot responses to soil resources are highly dynamic [36]. Plant dynamics are an exciting source of new traits for crop improvement because they can substantially reduce the energy

tropism, and initiation of root tips within a given soil [120].

Systems of systems: a way of thinking about plants in the environment that recognizes that the components and processes underlying each are interrelated and change in how one impacts the others; a way of thinking to accelerate development of a secure and sustainable plant-based food supply [121].

Trait: characteristic of an organism that may or may not be expressed in a phenotype and measured (phenotyped).

Trait value: the saving or gain (economic or material resources) arising from a trait; for example, thinner but longer roots increase N capture, saving N fertilizer application, saving the farmer money, and reducing environmental pollution. Validation and proof of concept of trait values may be used by breeders to invest (develop and market) a new cultivar with the trait.



Trends in Plant Science

Figure 2. Whole-Plant Phenotyping.

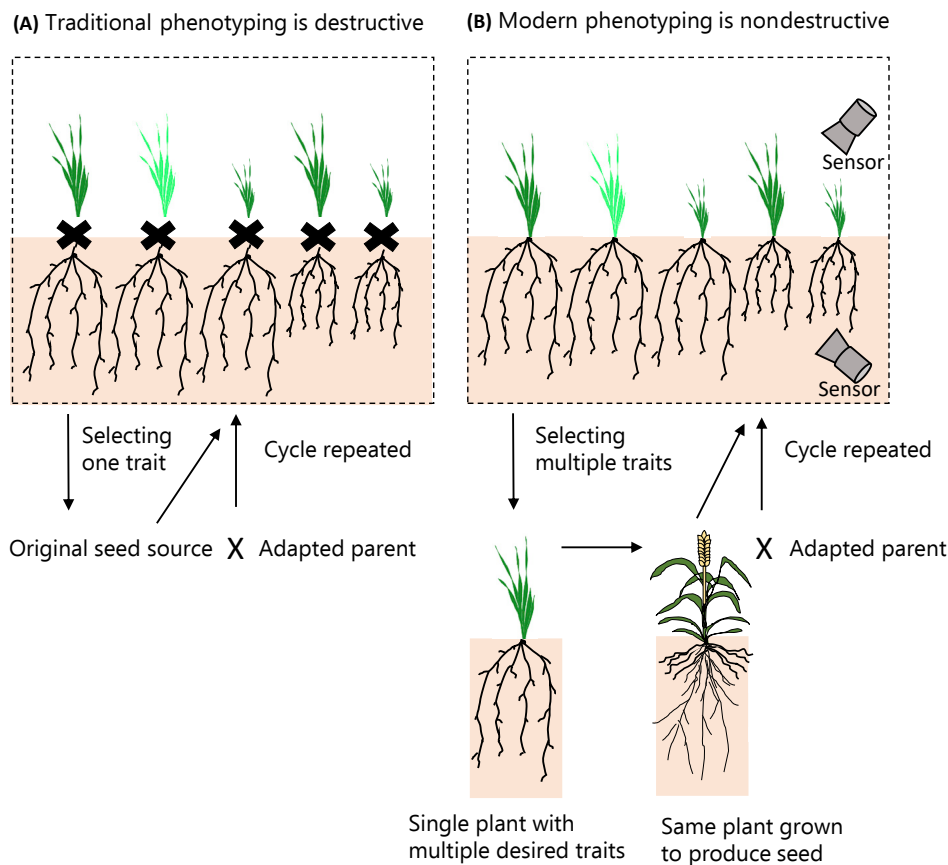
Noninvasive phenotyping offers simultaneous sensing of shoot and root growth and water movement in soil–plant systems. (A) Whole-plant, root–shoot phenotyping. Simultaneous phenotyping of shoots and roots using the automated rhizotron based platform GrowScreen-Rhizo [22] gives new insights into how root and shoot allocation is linked to the resource use efficiency of crop plants and enables whole-plant genetic gain. In this example, nitrogen starvation reduces the growth parameters of shoots of wheat, while it induces the opposite effect on root phenotypes. The drawing is based on phenotyping a collection of 36 wheat genotypes including wild emmer, emmer, and durums under optimal and nitrogen-starvation conditions [25]. (B) Simultaneous phenotyping of whole plants using magnetic resonance imaging (MRI) [44] allows temporal information about adventitious root growth from petunia cuttings for horticultural improvement [23]. The drawings depict phenotypic diversity of shoot (stem and leaves) and adventitious root growth in petunia cultivars, revealing the potential of technologies such as MRI to identify robust root development that is not apparent from shoot growth alone, for commercial use and breeding.

requirements of tolerance to soil stress [37]. Dynamics are challenging to phenotype because space and time dimensions are required. Phenotyping in 4D can be done with magnetic resonance imaging (MRI), X-ray computed tomography (CT), and positron emission tomography (PET) [38]. These technologies can measure root growth in different soil types and in undisturbed soil cores [39–41], and in response to phosphorus [42] and water [43]. Activation of meristematic activity in adventitious root development [23] (Figure 2B) and branch roots [44] have been quantified with MRI, and ^{11}C allocation in roots was detected with PET and co-registered with MRI images of the same plants [45].

Internationally available platforms have automated delivery of plants to imaging technologies for time lapse studies ([44], reviewed in [38] and [46]). The throughput of the available 4D root imaging technologies depends on pot size, soil type, detection method, and instrument automation and design. The exemplified MRI platform in Figure 2B can scan 350 small plants over 24 hours autonomously (14 plants per hour) [23]. Phenotyping is not only valuable once the trait is defined, it is highly valuable for discovering new traits through the activities of **deep phenotyping**. An emerging outcome of deep phenotyping is enhanced understanding of whole-plant physiology. (See Wasson *et al.* [47] for noninvasive root phenotyping technologies used to date, along the electromagnetic spectrum, with radiation strengths.)

Seed to Seed Noninvasive Phenotyping

Root phenotyping frequently involves destroying the plant (Figure 3A). Time is saved if the plant is phenotyped and then left to grow to seed. Delhaize and colleagues [48] took this approach with

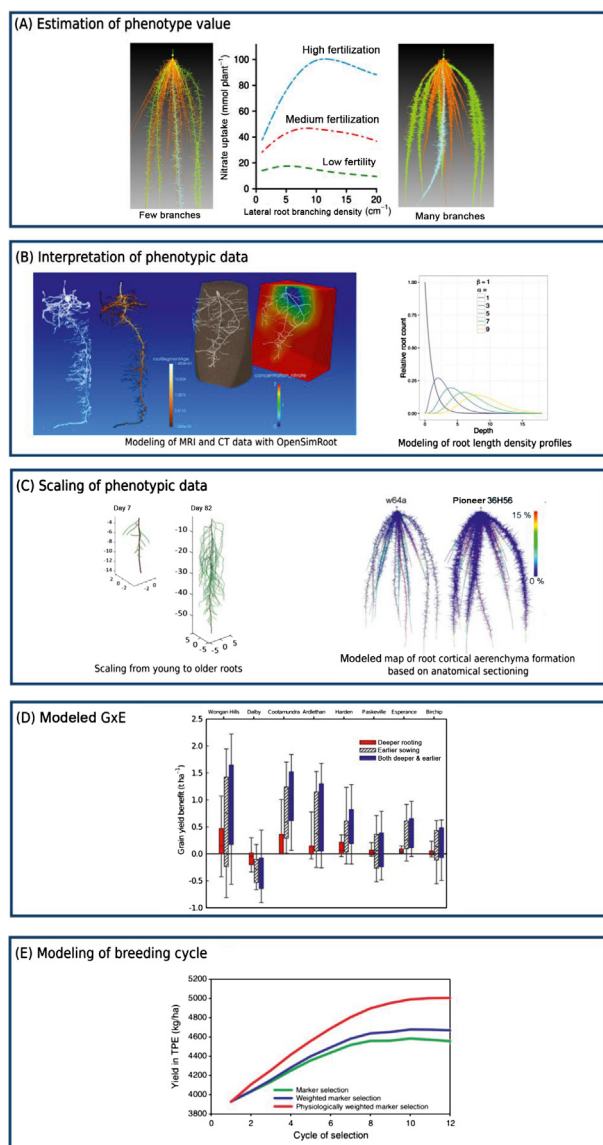


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Figure 3. Faster Selection and Pyramiding of Beneficial Traits with Nondestructive, Whole-Plant and Seed to Seed Phenotyping.

Traditional destructive phenotyping (A) contrasts with the flexibility and precision offered by nondestructive, noninvasive, multisensor, and multitrait phenotyping (B). (A) Traditional destructive methods do not allow combinations of developmental periods and dynamic environmental phenotypes, and new seed production is only possible by using the original genetic resources of the parental lines. (B) Noninvasive phenotyping allows traits to be measured over time on the same plants of populations generated from parental lines with multiple desired traits (such as high vigor, pathogen resistance, desired root and shoot traits combined in multiparent advanced generation intercross populations [122]). Phenotypic differences which occur transiently, at a certain developmental stage or only under certain environmental conditions, can be identified, combined, and pyramided on the same plant. After the selection of lines carrying desired traits, seed can be produced from the individuals because the plants are not destroyed during measurements.

rhizosheath phenotyping because nodal roots grew after seminal roots were cut for weighing. Noninvasive phenotyping technologies not only measure multiple traits in parallel (discussed above), they do so without destroying the plant over time, keeping seed viability [49]. Alleles of multiple desirable traits are advanced specifically in the seed expressing the traits (Figure 3B). At the stage of developing the parent line, shoot and root traits are combined in a desirable seed. This single seed can be followed through characterization of different plant stages; a young plant stage of desirable features can be combined with an older plant stage with desirable features, to select traits that scale more closely to yield [50]. Retaining the live seed of the line carrying all desired phenotypes is powerful at the stage of backcross breeding, which uses single-seed descent (shown in Figure 4 of [24]). Not only are desirable phenotypes advanced, undesirable features can be more quickly removed as generations advance.



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Figure 4. Mathematical Models and Their Contributions to Root and Shoot Phenotyping for Trait-Based Breeding.

(A) Estimation of phenotype value. The optimal root branching for N and P soil resources was tested in the model OpenSimRoot [88] and was used prior to a selection program to reduce treatments and measurements. (B) Interpretation of phenotypic data. Phenotyping roots in soil can generate high variation within and between samples. Structure–function models helped predict root architecture and nutrient uptake from images obtained noninvasively using magnetic resonance imaging (MRI) (far left) and X-ray computed tomography (CT) (middle) [83]. Bayesian statistics used with field core root distributions increased the heritability of deep root phenotypes (far right) [61]. (C) Scaling of phenotypic data. Phenotyping methods may be restricted to early plant stages. Models scale from young plant phenotypes to adult plants to better predict how the young will contribute to biomass at yield stages (left) [89] and estimate how much whole-plant biomass, root structure, and function will be influenced by cortical cell senescence that was phenotyped using microscopy on root sections (right) [86]. (D) Modeled genotype $\times$ environment $\times$ management (G $\times$ E $\times$ M). Phenotypes confer a value to the crop if the climate, soil, and management are suitable for trait expression. Crop models such as APSIMTM integrate climate and agronomic management practices over years and sites with phenotypes to test

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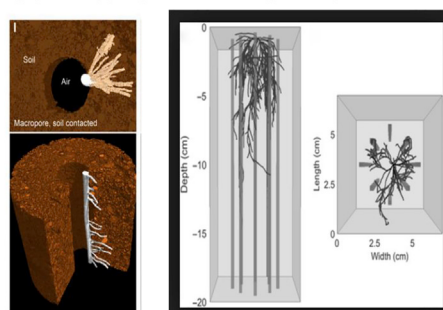
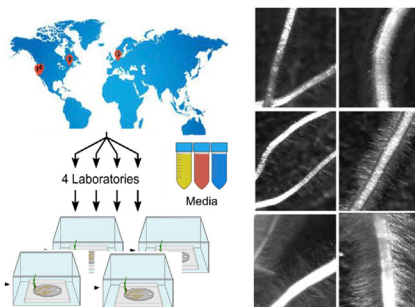
Field Phenotyping

A phenotypic screen with high repeatability and low variation is needed to gain root traits genetically. To date, controlled environments are more reliable for repeatable measurement of traits conferring productivity in regions with unpredictable climates [51]. A meta-analysis showed that shoot measurements in controlled environments have been more repeatable than those in fields [52]. The controlled environment offers higher chances of repeatable shoot, root, and dynamic phenotypes than field phenotyping for some phenotypes which show similar expression in controlled and field conditions; for example, seminal roots of young plants [53,54]. However, the controlled environment phenotype must have a significant impact on yield in the target field environment (see Figure 2 of [13]), and field phenotyping is a critical component of phenotype breeding. This is particularly the case for root system traits expressed during later stages in plant growth, in deep layers of the profile. Root system depth at the stage of grain development in wheat in the field is poorly represented in the laboratory [53] and rapid in-field canopy measurements such as temperature [55] and greenness [21] are much more reliable predictors of rooting depth than controlled environment screens.

Field phenotyping was used in all the examples in Figure 1. One main use was to select root phenotypes and germplasm sources directly in the field (e.g., [11,19–21]). Field techniques available today to select genotypes with desired root traits are invasive, minimally invasive, or indirect via shoots or soil (see [47] and reviews [56–58]). Coring [9], and ‘shovelomics’ [59] are widely used invasive methods (see Figure 2 of [47] for contrast between these methods). Both methods have a throughput comparable with the rhizotron and MRI systems highlighted in Figure 2: per hour, 15 cores can be taken and imaged with three people [60] and eight plots can be shoveled, washed, and imaged per person (L. York, personal communication). Coring, in-field core-break, in-field automated imaging of core faces [60], and Bayesian hierarchical nonlinear mixed modeling, provide root counts in soil over soil depths, which can be treated as a single heritable function [61]. Shovelomics quantifies traits on the washed ‘root crown’. Root number, growth, and angle were measured in various species and soil conditions [62–65]. Digital imaging of root traits, root estimator for shovelomics traits, and the multiperspective imaging platform [66–68] standardize and increase the root measurements captured from multiple images of the same root crown. Both coring and shovelomics offer substantial opportunities when used with postprocessing to discover or validate traits. Apparatus have been developed for washing core fragments [69] and quantifying root length [70]. Roots and/or soil can be analyzed for root DNA [71] and root anatomy [72]. Shovelomics has been combined with X-ray CT [73]. Mini-rhizotrons, unlike coring and shovelomics, are inserted and left in place prior to arrival of roots. The plane between the tube and the soil becomes a microenvironment that can alter root growth [74] and hence they are considered minimally invasive (see second figure, [47]). Modern mini-rhizotron imaging systems have automated cameras with positioning systems and image analysis [75]. They have identified genotypic variation to response to irrigation [76], deep root growth and functional variation among species and genotypes within species [77], and biotic interactions with roots, including nodule development in response to above-ground CO₂ [78]. Andrew Leakey and colleagues installed 3000 access tubes to collect 330 000 images of 750 maize hybrids: a paradigm of mini-rhizotron use in phenotyping (<https://twitter.com/leakey77/status/1026833803921301504>).

Another main use of field phenotyping is to quantify contribution of the trait to yield, yield stability, resource use efficiency, and, ultimately, profitability in given field conditions. In particular, breeders need proof of concept and a **trait value** estimate to decide whether to invest the time and resources to develop cultivars for release [79]. Trait value validation is carried out in target field environments and therefore relies on excellent field phenotyping of shoots, roots, and yield products. Germplasm

phenotypic value and expression [90] and simulated that deeper rooting consistently resulted in greater yields in seven out of eight Australian farming systems, and that earlier sowing management was much more valuable for deeper roots than potential genetic variation. (E) Modeling of breeding cycle. Modeling demonstrated that phenotype information increased the rate of yield gain in breeding cycles over marker selection and weighted marker selection alone [91].

(A) *In situ* field discovery**(B) Deep phenotyping and modeling field phenomena****(C) Repeatable microcosms to test multiple rhizosphere components**

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Figure 5. Research Approaches to Discover New Root–Soil Rhizosphere Phenotypes for the Future.

The challenge for crop improvement using root traits is discovering and selecting for interactions with heterogeneous field conditions. (A) *In situ* field discovery shows that roots are influenced by combinations of abiotic and biotic factors. Left: root tips in the field have variable morphological distortions and soil binding (upper images) and abundance of bacteria (compare bright points on lower images, which correspond to tips above) [123]. Right: field roots have variable attachments to dead roots of previous crops in biopores. Image shows pale crop root with dead roots on right and soil on left, within a pore space (provided by Margaret McCully). (B) Deep phenotyping and modeling complex field phenomena. Left: micro-X-ray computed tomography combined with modeling shows the strong effect of soil contact and associated moisture on root structure, mediated by branching and underlying genes [105]. Right: model to test how biopore factors influence root growth [104]. (C) Recombining field factors using repeatable, multi-lab microcosms. Testable hypotheses require repeatable experimental systems that can be scaled to many experiments to be able to combine the many factors found in the field. Left: the EcoFAB developed in the Northern group generates repeatable

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that is compared has varied in different prebreeding programs. In order of increasing precision, resources, and time for development, comparisons have been between: lines contrasting for phenotype proxy [80] plus other phenotypes; 'tails' with the trait [79] in a range of backgrounds; and near isogenic lines that are made with the trait in an adapted background [81]. One proposed method to speed up trait introgression and validation is to integrate field and controlled environment at the start of a phenotyping program [24].

Simulation Models Aid the Use of Phenotyping for Discovery and Trait-Based Breeding and Agronomy

Modeling is an expanding area of crop science, to integrate scales and systems of research [82]. Here, we present **models** available now to aid phenotype and trait-based breeding of root systems suited to climates and farming practices (Figure 4). Root architecture models open to researchers such as OpenSimRoot [83] can test trait synergisms (Figure 4A) (e.g., [84]), helping to estimate the value of co-selection of phenotypes using technologies highlighted in Figure 2, and their value to yield across environments [85]. OpenSimRoot can also model root images obtained from noninvasive scanning in soil using MRI and X-ray CT (Figure 4B) [83]. Mechanistic models of plant growth and rhizosphere processes have been used to study phenotypes and their value in different soil conditions [86,87]. Simulated responses to concentrations of resources can greatly narrow down the number of observations and testing environments required for a phenotyping program [88]. New statistical models have been employed to connect field root observations to traits, where simple linear correlations and methods that rely on normal distributions fail (Figure 4B) [61]. Once constructed, the models may be used to predict the relationship of a phenotype beyond a defined plant age and phenological stage (Figure 4C) [89]. At the agronomic and breeding scales, models are extremely valuable to help design cross year and environment activities (Figure 4D). The value from deep rooting in rain-fed rotation systems was modeled across 100 years of climatic data [90] and the value of combining physiological trait selection within breeding cycles was modeled [91] (Figure 4E).

Where to Next: Fields of Discovery in the Rhizosphere that Includes the Root

Productivity of crops on farms is the result of a combination of genetics, environment, and management. Excellent examples and reviews show that future productivity can be achieved faster than in the past by integrating disciplines [92,93]. Past genetic and management gains in farming have been associated with roots and soil management (tillage, fertilizer additions, and rotations) and we propose more in future by making discoveries in fields (Figure 5). The rhizosphere is the key space for discovery of new root traits that are relevant and valuable to fields and farming systems.

York *et al.* [94] suggest treating the soil influenced by the root in terms of biological, structural, and chemical changes, for a **rhizosphere phenotype**. We extend the spatial and temporal definition of the rhizosphere further than that proposed by York and colleagues, to include the associated root. Elegant recent research shows that root cap cells signal back to the root to influence branch root emergence through a program of cell death, once detached and left along the root [95]. We anticipate that this holistic rhizosphere definition that includes the root will speed up direct selection of root traits within farm and soil management systems. Phenotyping the rhizosphere, including the root, retains the conditions for trait expression [96] and greatly increases the chances of discovering new phenotypes for root function, such as in the case of the rhizosheath trait and its association with root hairs [48]. Malate exudation for aluminum tolerance is an example of a rhizosphere phenotype [14]. However, its translation to increased plant growth depends on soil conditions [97]. Soil conditions influence the effectiveness of the exudate phytate [98] and, more critically, the soil

root, shoot, and exudate phenotypes across laboratories internationally. EcoFABs are 3D printed, scalable, and axenic, allowing multiple soil factors to be tested individually and in combination. Right: the EcoFABs showed that root hair development is influenced by filtered soil extracts differently to P supply [109]. Abbreviations: E, root epidermis; n, nucleus; rh, roothair.

conditions plus the root zone of citrate efflux was important for citrate contribution to plant P uptake [99].

The boundary between the root and the soil is not sharp in fields (Figure 5A) and depends, to name a few factors, on root growth rate, biopores, position along roots, and contact with decaying root organic matter. It is doubtful that the anatomy of the root–soil interface mucigel with cap cells and microorganisms can be dissected spatially: it is a mixture of root and microbiota polysaccharides, root cap cells, and soil particles (see micrographs in [100]). Rhizosphere phenotyping methods (see thorough review in [101]) can be used for *in situ* field discovery of new holistic rhizosphere phenotypes. For example, soil zymography quantifies exuded plant and microbial enzymes [102], and planar optodes detect pH and oxygen changes around roots in soil [103]. Field phenomena can be tested with deep phenotyping, modeling, and genetics (Figure 5B). Biopore environments are very different environments for roots than the bulk soil (Figure 5A) and effort is going into predictive modeling of root growth into them [104] (Figure 5B). Genetic regulators of growth patterns of roots along the edges of pores, mediated by moisture, was simulated and tested (Figure 5B, right) [105]. X-ray CT detectors allow the rhizosphere fine physical structure to be measured as it is altered by the root *in situ* in soil, along with predictions on water and nutrient uptake [87,106–108]. But how can the large number of variables in the rhizosphere be systematically combined for the understanding of processes? An exciting multi-lab approach has been initiated, using repeatable, 3D printed microcosms called Eco-Fabs (Figure 5C) [109]. Bespoke phenotyping platforms are available globally in fixed locations with highly specialized operators for researchers, breeders, and prebreeders [46]. It is predicted that mobile, easy to build systems to test cross-lab repeatability, will be a new addition to plant and rhizosphere phenotyping in future.

Concluding Remarks and Future Perspectives

Retrospective analysis of trait-based root improvement to date (Figure 1) reveals great successes, but opportunities will be faster with today's technologies and with approaches in thinking and actions that integrate the rhizosphere and breeding and agronomy. An analysis for traits selected for water-limited cereals also found that the time between phenotype conception to proof of concept with germplasm for breeders was too long to meet the demands of food security [110]. There are exciting rhizosphere phenotypes available for integrated programs; for example, NUE from biological nitrification inhibitor root exudates [111] and the regulation of branch root architecture by water availability [105]. Past examples suggest that these single traits should be co-selected with desirable above-ground phenotypes and within soil and management conditions to be used on farms.

We expect in future that phenotyping technologies will be used on farmers' fields to quantify the integrated phenotype of the crop due to management practices, soil inputs, and breeding. Phenotyping rhizospheres could be combined with high-throughput canopy phenotyping data, highly useful in soybean and wheat crops to identify seedling and mature stages [112]. Further pattern recognition software can be used for new algorithms to identify combined rhizosphere and shoot phenotypes associated with beneficial traits for selection or management [113]. A new initiative has been established to attempt to harness crop-management synergies using phenotyping, robotics, and computational technologies (<http://www.phenorob.de/>).

Shoot and rhizosphere phenotyping in combination with precision agriculture may allow selection of genotypes and practices on farms to speed up productivity gains. However, this requires a substantial shift in the approach, timelines, and intensities of activities of research programs and a change in the genotype to phenotype paradigm. Technologies for phenotyping are continually expanding [114], driving the expansion of what is measured as the phenotype. Similarly, genomic technologies are driving the boundaries of the genotype, revealing epigenetics [115] and plants hosting microbiomes as **holobionts** [116]. Expanding terms and definitions for a given context will allow phenotyping to move forward, continue to play multiple and diverse roles within breeding and agronomy, and generate collaboration and opportunities in new disciplines and across industries (see Outstanding Questions).

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References

- O'Toole, J.C. and Bland, W.L. (1987) Genotypic variation in crop plant root systems. *Adv. Agron.* 41, 91–145
- Watt, M. et al. (2006) Rhizosphere biology and crop productivity - a review. *Aust. J. Soil Res.* 44, 299–317
- Gregory, P.J. et al. (2009) Root phenomics of crops: opportunities and challenges. *Funct. Plant Biol.* 36, 922–929
- Kuijken, R.C.P. et al. (2015) Root phenotyping: from component trait in the lab to breeding. *J. Exp. Bot.* 66, 5389–5401
- Chochois, V. et al. (2012) Application of *Brachypodium* to the genetic improvement of wheat roots. *J. Exp. Bot.* 63, 3467–3474
- Negin, B. and Moshelion, M. (2017) The advantages of functional phenotyping in pre-field screening for drought-tolerant crops. *Funct. Plant Biol.* 44, 107–118
- Hurd, E.A. (1974) Phenotype and drought tolerance in wheat. *Agr. Meteorol.* 14, 39–55
- Uga, Y. et al. (2011) Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *J. Exp. Bot.* 62, 2485–2494
- Wasson, A.P. et al. (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
- Richards, R.A. and Passioura, J.B. (1989) A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain-yield in rain-fed environments. *Aust. J. Agr. Res.* 40, 943–950
- Caradus, J.R. and Woodfield, D.R. (1998) Genetic control of adaptive root characteristics in white clover. *Plant Soil* 200, 63–69
- Henry, A. et al. (2010) Will nutrient-efficient genotypes mine the soil? Effects of genetic differences in root architecture in common bean (*Phaseolus vulgaris* L.) on soil phosphorus depletion in a low-input agro-ecosystem in Central America. *Field Crop Res.* 115, 67–78
- Wissuwa, M. et al. (2016) From promise to application: root traits for enhanced nutrient capture in rice breeding. *J. Exp. Bot.* 67, 3605–3615
- Sasaki, T. et al. (2005) Overexpression of wheat ALMT1 gene confers aluminum tolerance in plants. *Plant Cell Physiol.* 46, S158
- Munns, R. et al. (2012) Wheat grain yield on saline soils is improved by an ancestral Na⁺ transporter gene. *Nat. Biotechnol.* 30, 360–364
- Ogbonnaya, F.C. et al. (2001) Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. *Aust. J. Agr. Res.* 52, 1367–1374
- Schroeder, J.I. et al. (2013) Using membrane transporters to improve crops for sustainable food production. *Nature* 497, 60–66
- Pinto, R.S. and Reynolds, M.P. (2015) Common genetic basis for canopy temperature depression under heat and drought stress associated with optimized root distribution in bread wheat. *Theor. Appl. Genet.* 128, 575–585
- Pask, A. et al. (2014) A wheat phenotyping network to incorporate physiological traits for climate change in South Asia. *Field Crop Res.* 168, 156–167
- Rich, S.M. et al. (2016) Wheats developed for high yield on stored soil moisture have deep vigorous root systems. *Funct. Plant Biol.* 43, 173–188
- Li, X. et al. (2019) Deeper roots associated with cooler canopies, higher normalized difference vegetation index, and greater yield in three wheat populations grown on stored soil water. *J. Exp. Bot.* 70, 4963–4974
- Nagel, K.A. et al. (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
- Jansen, M. et al. (2014) Non-invasive phenotyping methodologies enable the accurate characterization of growth and performance of shoots and roots. In *Genomics of Plant Genetic Resources* (Tuberosa, R. et al. eds), pp. 173–206, Springer
- Wasson, A.P. et al. (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *J. Exp. Bot.* 63, 3485–3498
- Gioia, T. et al. (2015) Impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilization. *J. Exp. Bot.* 66, 5519–5530
- Chochois, V. et al. (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
- Nagel, K.A. et al. (2015) Simultaneous effects of leaf irradiance and soil moisture on growth and root system architecture of novel wheat genotypes: implications for phenotyping. *J. Exp. Bot.* 66, 5441–5452
- Pfeifer, J. et al. (2014) Spring barley shows dynamic compensatory root and shoot growth responses when exposed to localised soil compaction and fertilisation. *Funct. Plant Biol.* 41, 581–597
- Achim, W. and Nagel, K.A. (2006) Root growth reacts rapidly and more pronounced than shoot growth towards increasing light intensity in tobacco seedlings. *Plant Signal. Behav.* 1, 225–226
- Hecht, V.L. et al. (2016) Sowing density: a neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (*Hordeum vulgare* L.). *Front. Plant Sci.* 7, 944
- Barboza-Barquero, L. et al. (2015) Phenotype of *Arabidopsis thaliana* semi-dwarfs with deep roots and high growth rates under water-limiting conditions is independent of the GA5 loss-of-function alleles. *Ann. Bot.* 116, 321–331
- Gioia, T. et al. (2016) GrowScreen-PaGe, a non-invasive, high-throughput phenotyping system based on germination paper to quantify crop phenotypic diversity and plasticity of root traits under varying nutrient supply. *Funct. Plant Biol.* 44, 76–93
- Avramova, V. et al. (2016) Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *J. Exp. Bot.* 67, 2453–2466

Outstanding Questions

What are the boundaries of a root phenotype, in terms of soil and shoots?

Can the dynamics of root responses in soil be exploited to increase agricultural productivity, requiring the exploitation of large amounts of dynamic phenotypic data in breeding and on farms?

Is the timing correct to exploit the knowledge that pedoclimate and management are the important determinants of yield, through both root and shoot traits?

How do plants sense and respond to the combination of factors in the environment?

How do scientists shift from single factor hypotheses to combinatorial hypotheses and experiments?

34. Nakhforoosh, A. et al. (2016) Identification of water use strategies at early growth stages in durum wheat from shoot phenotyping and physiological measurements. *Front. Plant Sci.* 7, 1155
35. Al-Tamimi, N. et al. (2016) Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nat. Commun.* 7, 13342
36. Schurr, U. et al. (2006) Functional dynamics of plant growth and photosynthesis – from steady-state to dynamics – from homogeneity to heterogeneity. *Plant, Cell Environ.* 29, 340–352
37. Arsova, B. et al. (2019) Dynamics in plant roots and shoots minimise stress, save energy and maintain water and nutrient uptake. *New Phytol.* Published online May 24, 2019. <https://doi.org/10.1111/nph.15955>
38. Atkinson, J.A. et al. (2019) Uncovering the hidden half of plants using new advances in root phenotyping. *Curr. Opin. Biotechnol.* 55, 1–8
39. Metzner, R. et al. (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
40. Pflugfelder, D. et al. (2017) Non-invasive imaging of plant roots in different soils using magnetic resonance imaging (MRI). *Plant Methods* 13, 102
41. Tracy, S.R. et al. (2010) The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. *J. Exp. Bot.* 61, 311–313
42. Flavel, R.J. et al. (2014) Quantifying the response of wheat (*Triticum aestivum* L.) root system architecture to phosphorus in an Oxisol. *Plant Soil* 385, 303–310
43. Dietrich, D. (2018) Hydrotropism: how roots search for water. *J. Exp. Bot.* 69, 2759–2771
44. van Dusschoten, D. et al. (2016) Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. *Plant Physiol.* 170, 1176–1188
45. Jahnke, S. et al. (2009) Combined MRI-PET dissects dynamic changes in plant structures and functions. *Plant J.* 59, 634–644
46. Pieruschka, R. and Schurr, U. (2019) Plant phenotyping: past, present, and future. *Plant Phenomics* 2019, 6
47. Wasson, A.P. et al. (2019) Beyond digging: non-invasive root and rhizosphere phenotyping. *Trends Plant Sci.* 25, <https://doi.org/10.1016/j.tplants.2019.10.011>
48. Delhaize, E. et al. (2012) Aluminium tolerance of root hairs underlies genotypic differences in rhizosheath size of wheat (*Triticum aestivum*) grown on acid soil. *New Phytol.* 195, 609–619
49. Jahnke, S. et al. (2016) PhenoSeeder - a robot system for automated handling and phenotyping of individual seeds. *Plant Physiol.* 172, 1358
50. Sadras, V.O. and Richards, R.A. (2014) Improvement of crop yield in dry environments: benchmarks, levels of organisation and the role of nitrogen. *J. Exp. Bot.* 65, 1981–1995
51. Richards, R.A. et al. (2010) Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. *Funct. Plant Biol.* 37, 85–97
52. Poorter, H. et al. (2016) Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol.* 212, 838–855
53. Watt, M. et al. (2013) A rapid, controlled-environment seedling root screen for wheat correlates well with rooting depths at vegetative, but not reproductive, stages at two field sites. *Ann. Bot.* 112, 447–455
54. Maccaferri, M. et al. (2016) Prioritizing quantitative trait loci for root system architecture in tetraploid wheat. *J. Exp. Bot.* 67, 1161–1178
55. Lopes, M.S. and Reynolds, M.P. (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37, 147–156
56. Wasaya, A. et al. (2018) Root phenotyping for drought tolerance: a review. *Agronomy* 8, 241
57. Araus, J.L. and Cairns, J.E. (2014) Field high-throughput phenotyping: the new crop breeding frontier. *Trends Plant Sci.* 19, 52–61
58. Whalley, W.R. et al. (2017) Methods to estimate changes in soil water for phenotyping root activity in the field. *Plant Soil* 415, 407–422
59. Trachsel, S. et al. (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* 341, 75–87
60. Wasson, A. et al. (2016) A portable fluorescence spectroscopy imaging system for automated root phenotyping in soil cores in the field. *J. Exp. Bot.* 67, 1033–1043
61. Wasson, A.P. et al. (2017) Differentiating wheat genotypes by Bayesian hierarchical nonlinear mixed modeling of wheat root density. *Front. Plant Sci.* 8, 282
62. York, L.M. and Lynch, J.P. (2015) Intensive field phenotyping of maize (*Zea mays* L.) root crowns identifies phenes and phene integration associated with plant growth and nitrogen acquisition. *J. Exp. Bot.* 66, 5493–5505
63. Sun, B. et al. (2018) Large crown root number improves topsoil foraging and phosphorus acquisition. *Plant Physiol.* 177, 90
64. Slack, S. et al. (2018) Wheat shovelomics II: revealing relationships between root crown traits and crop growth. *bioRxiv*. Published online March 13, 2018. <https://doi.org/10.1101/280917>
65. Burrige, J. et al. (2016) Legume shovelomics: high-throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp. *unguiculata*) root architecture in the field. *Field Crops Res.* 192, 21–32
66. Bucksch, A. et al. (2014) Image-based high-throughput field phenotyping of crop roots. *Plant Physiol.* 166, 470
67. Colombi, T. et al. (2015) Next generation shovelomics: set up a tent and REST. *Plant Soil* 388, 1–20
68. Seethepalli, A. et al. (2018) A novel multi-perspective imaging platform (M-PIP) for phenotyping soybean root crowns in the field increases throughput and separation ability of genotype root properties. *bioRxiv*. Published online May 2, 2018. <https://doi.org/10.1101/309161>
69. Smucker, A.J.M. et al. (1982) Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy J.* 74, 500–503
70. Delory, B.M. et al. (2017) Accuracy and bias of methods used for root length measurements in functional root research. *Methods in Ecology and Evolution* 8, 1594–1606
71. Huang, C.Y. et al. (2013) A DNA-based method for studying root responses to drought in field-grown wheat genotypes. *Sci. Rep.* 3, 3194
72. Watt, M. et al. (2008) Types, structure and potential for axial water flow in the deepest roots of field-grown cereals. *New Phytol.* 178, 690
73. Jiang, N. et al. (2019) High-resolution 4D spatiotemporal analysis reveals the contributions of local growth dynamics to contrasting maize root architectures. *Plant Cell* 31, 1708–1722

74. Rytter, R.-M. and Rytter, L. (2012) Quantitative estimates of root densities at minirhizotrons differ from those in the bulk soil. *Plant Soil* 350, 205–220
75. Vamerali, T. et al. (2011) Minirhizotrons in modern root studies. In *Measuring Roots* (Mancuso, S. ed), pp. 341–361, Springer
76. Ohashi, A.Y.P. et al. (2015) Root growth and distribution in sugarcane cultivars fertigated by a subsurface drip system. *Bragantia* 74, 131–138
77. Chen, S. et al. (2019) Evaluation of deep root phenotyping techniques in tube rhizotrons. *Acta Agric. Scand. B Plant Sci.* 69, 62–74
78. Gray, S.B. et al. (2013) Minirhizotron imaging reveals that nodulation of field-grown soybean is enhanced by free-air CO₂ enrichment only when combined with drought stress. *Funct. Plant Biol.* 40, 137–147
79. Rebetzke, G.J. et al. (2012) A multisite managed environment facility for targeted trait and germplasm phenotyping. *Funct. Plant Biol.* 40, 1–13
80. Chimungu, J.G. et al. (2014) Large root cortical cell size improves drought tolerance in maize. *Plant Physiol.* 166, 2166
81. James, R.A. et al. (2016) Rhizosheaths on wheat grown in acid soils: phosphorus acquisition efficiency and genetic control. *J. Exp. Bot.* 67, 3709–3718
82. Marshall-Colon, A. et al. (2017) Crops in silico: generating virtual crops using an integrative and multi-scale modeling platform. *Front. Plant Sci.* 8, 786
83. Postma, J.A. et al. (2017) OpenSimRoot: widening the scope and application of root architectural models. *New Phytol.* 215, 1274–1286
84. Rangarajan, H. et al. (2018) Co-optimization of axial root phenotypes for nitrogen and phosphorus acquisition in common bean. *Ann. Bot.* 122, 485–499
85. Hoogenboom, G. et al. (2004) From genome to crop: integration through simulation modeling. *Field Crops Res.* 90, 145–163
86. Schneider, H.M. et al. (2017) Root cortical senescence improves growth under suboptimal availability of N, P, and K. *Plant Physiol.* 174, 2333
87. Daly, K.R. et al. (2017) Quantification of root water uptake in soil using X-ray computed tomography and image-based modelling. *Plant Cell Environ.* 41, 121–133
88. Postma, J.A. et al. (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. *Plant Physiol.* 166, 590
89. Zhao, J. et al. (2017) Root architecture simulation improves the inference from seedling root phenotyping towards mature root systems. *J. Exp. Bot.* 68, 965–982
90. Lilley, J.M. and Kirkegaard, J.A. (2016) Farming system context drives the value of deep wheat roots in semi-arid environments. *J. Exp. Bot.* 67, 3665–3681
91. Hammer, G.L. et al. (2005) Trait physiology and crop modelling as a framework to link phenotypic complexity to underlying genetic systems. *Aust. J. Agric. Res.* 56, 947–960
92. Kirkegaard, J.A. and Hunt, J.R. (2010) Increasing productivity by matching farming system management and genotype in water-limited environments. *J. Exp. Bot.* 61, 4129–4143
93. Hatfield, J.L. and Walthall, C.L. (2015) Meeting global food needs: realizing the potential via genetics × environment × management interactions. *Agronomy J.* 107, 1215–1226
94. York, L.M. et al. (2016) The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. *J. Exp. Bot.* 67, 3629–3643
95. Xuan, W. et al. (2016) Cyclic programmed cell death stimulates hormone signaling and root development in *Arabidopsis*. *Science* 351, 384
96. Watt, M. et al. (2006) Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environ. Microbiol.* 8, 871–884
97. Scheffe, C.R. et al. (2008) Organic anions in the rhizosphere of Al-tolerant and Al-sensitive wheat lines grown in an acid soil in controlled and field environments. *Aust. J. Soil Res.* 46, 257–264
98. George, T.S. et al. (2005) Expression of a fungal phytase gene in *Nicotiana tabacum* improves phosphorus nutrition of plants grown in amended soils. *Plant Biotechnol. J.* 3, 129–140
99. Ryan, P.R. et al. (2014) Can citrate efflux from roots improve phosphorus uptake by plants? Testing the hypothesis with near-isogenic lines of wheat. *Physiol. Plant.* 151, 230–242
100. Foster, R.C. (1981) The ultrastructure and histochemistry of the rhizosphere. *New Phytol.* 89, 263–273
101. Oburger, E. and Schmidt, H. (2016) New methods to unravel rhizosphere processes. *Trends Plant Sci.* 21, 243–255
102. Spohn, M. et al. (2013) Soil zymography – a novel in situ method for mapping distribution of enzyme activity in soil. *Soil Biol. Biochem.* 58, 275–280
103. Faget, M. et al. (2013) Disentangling who is who during rhizosphere acidification in root interactions: combining fluorescence with optode techniques. *Front. Plant Sci.* 4, 392
104. Landl, M. et al. (2017) A new model for root growth in soil with macropores. *Plant Soil* 415, 99–116
105. Bao, Y. et al. (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9319
106. Daly, K.R. et al. (2015) Assessing the influence of the rhizosphere on soil hydraulic properties using X-ray computed tomography and numerical modelling. *J. Exp. Bot.* 66, 2305–2314
107. Helliwell, J.R. et al. (2017) The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. *Sci. Rep.* 7, 14875
108. Rabbi, S.M.F. et al. (2018) Plant roots redesign the rhizosphere to alter the three-dimensional physical architecture and water dynamics. *New Phytol.* 219, 542–550
109. Sasse, J. et al. (2019) Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. *New Phytol.* 222, 1149–1160
110. Hall, A.J. and Richards, R.A. (2013) Prognosis for genetic improvement of yield potential and water-limited yield of major grain crops. *Field Crops Res.* 143, 18–33
111. O'Sullivan, C.A. et al. (2016) Identification of several wheat landraces with biological nitrification inhibition capacity. *Plant Soil* 404, 61–74
112. Bai, G. et al. (2016) A multi-sensor system for high throughput field phenotyping in soybean and wheat breeding. *Comput. Electron. Agr.* 128, 181–192
113. Mohanty, S.P. et al. (2016) Using deep learning for image-based plant disease detection. *Front. Plant Sci.* 7, 1419

114. Fiorani, F. and Schurr, U. (2013) Future scenarios for plant phenotyping. *Annu. Rev. Plant Biol.* 64, 267–291
115. Quadrana, L. and Colot, V. (2016) Plant transgenerational epigenetics. *Annu. Rev. Genet.* 50, 467–491
116. Vandenkoornhuyse, P. et al. (2015) The importance of the microbiome of the plant holobiont. *New Phytol.* 206, 1196–1206
117. Robinson, P.N. (2012) Deep phenotyping for precision medicine. *Hum. Mutat.* 33, 777–780
118. Hartmann, A. et al. (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil* 312, 7–14
119. Lynch, J. (1995) Root architecture and plant productivity. *Plant Physiol.* 109, 7–13
120. Rich, S.M. and Watt, M. (2013) Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. *J. Exp. Bot.* 64, 1193–1208
121. Giller, K. et al. (2018) *Developing Global Priorities for Plant Research*, Supporters of Agricultural Research, <https://supportagresearch.org/our-projects/developing-global-priorities-for-plant-research>
122. Huang, B.E. et al. (2015) MAGIC populations in crops: current status and future prospects. *Theor. Appl. Genet.* 128, 999–1017
123. Watt, M. et al. (2003) Soil strength and rate of root elongation alter the accumulation of *Pseudomonas* spp. and other bacteria in the rhizosphere of wheat. *Funct. Plant Biol.* 30, 483–491