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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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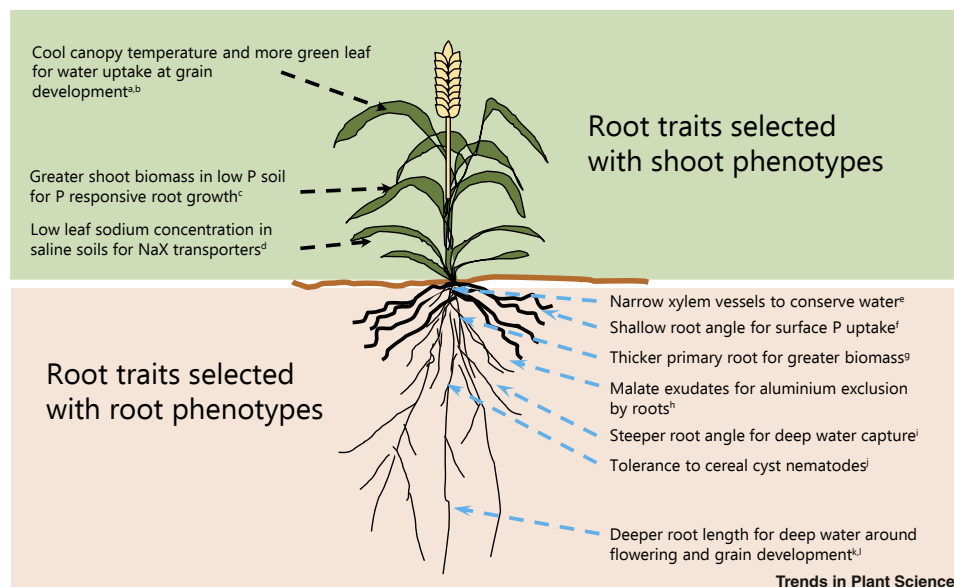
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Key Figure

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

Cool canopy temperature and more green leaf for water uptake at grain development[a,b]

Greater shoot biomass in low P soil for P responsive root growth[c]

Low leaf sodium concentration in saline soils for NaX transporters[d]

Root traits selected with shoot phenotypes

Root traits selected with root phenotypes

Narrow xylem vessels to conserve water[e]
Shallow root angle for surface P uptake[f]
Thicker primary root for greater biomass[g]
Malate exudates for aluminium exclusion by roots[h]
Steeper root angle for deep water capture[i]
Tolerance to cereal cyst nematodes[j]

Deeper root length for deep water around flowering and grain development[k,l]

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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 soil[i] [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

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.
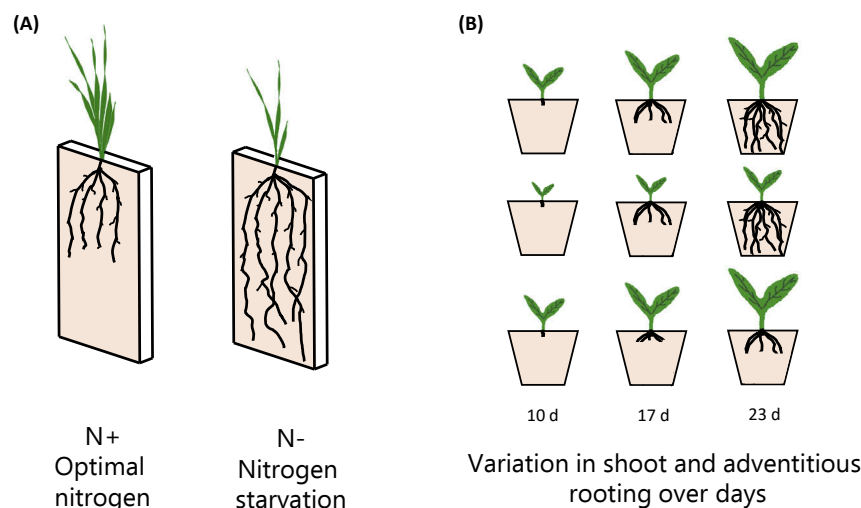
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Figure 2. Whole-Plant Phenotyping.
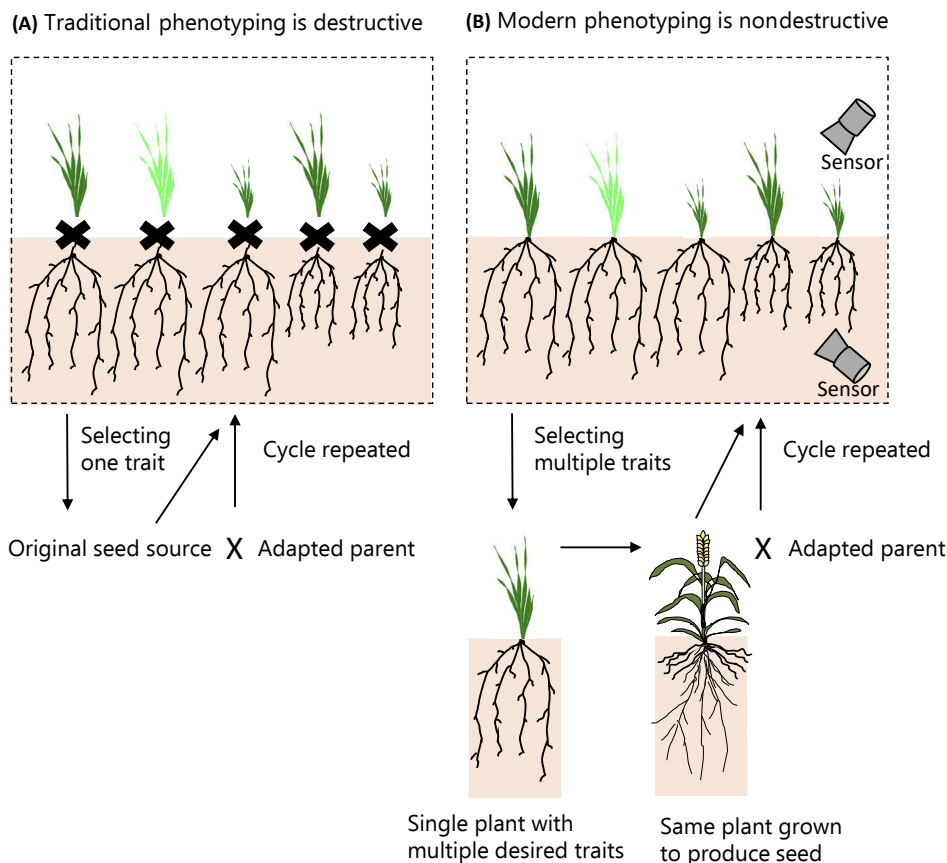Noninvasive phenotyping offers simultaneous sensing of shoot and root growth and water movement in soil–plant systems. (A) Whole-plant, root–shoot phenotyping. Simultaneous phenotyping of shoots and roots using the automated rhizotron based platform GrowScreen-Rhizo [22] gives new insights into how root 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

(A) Traditional phenotyping is destructive   **(B)** Modern phenotyping is nondestructive

Sensor

Sensor

Selecting one trait   Cycle repeated   Selecting multiple traits   Cycle repeated

Original seed source **X** Adapted parent

Single plant with multiple desired traits   **X** Adapted parent

Same plant grown to produce seed

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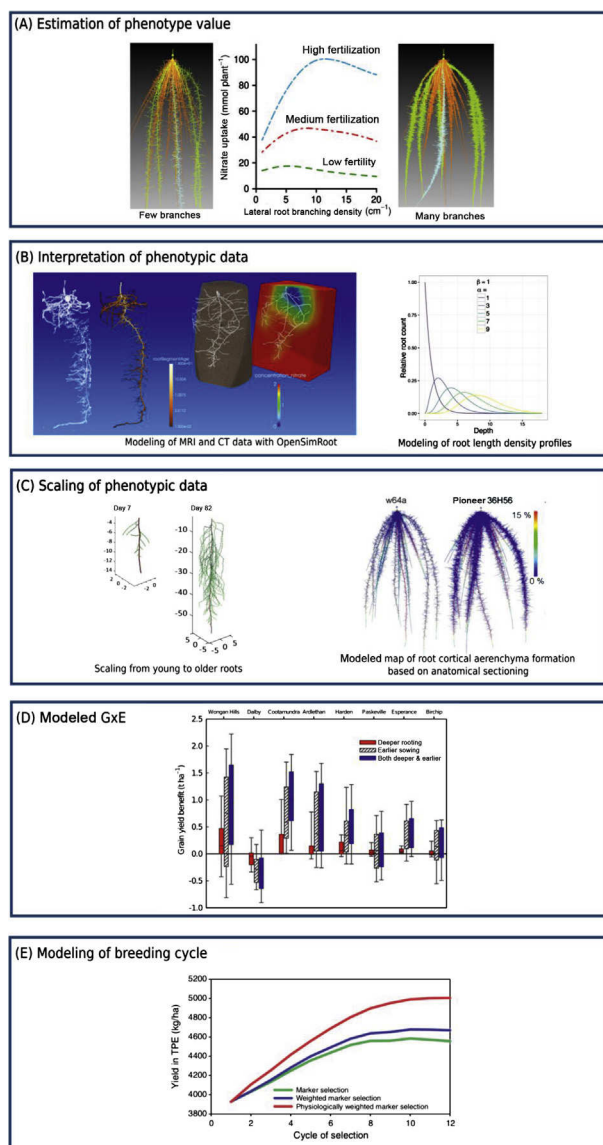
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Figure 4. Mathematical Models and Their Contributions to Root and Shoot Phenotyping for Trait-Based Breeding.

(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 × environment × management (G × E × M). Phenotypes confer a value to the crop if the climate, soil, and management are suitable for trait expression. Crop models such as APSIM™ integrate climate and agronomic management practices over years and sites with phenotypes to test

(Figure legend continued at the bottom of the next page.)

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 CO_2 [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

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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
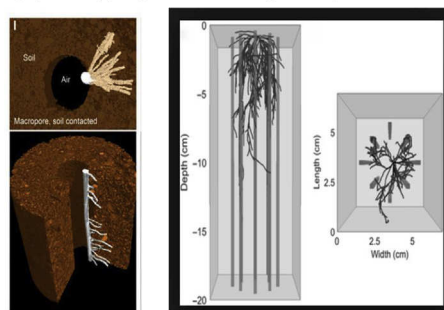
(C) Repeatable microcosms to test multiple rhizosphere components
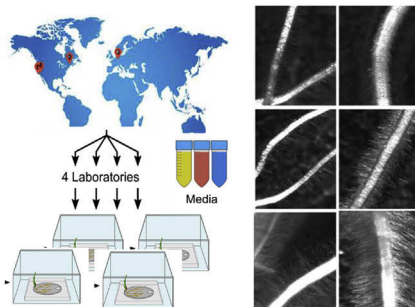
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Figure 5. Research Approaches to Discover New Root–Soil Rhizosphere Phenotypes for the Future.
The challenge for crop improvement using root traits is discovering and selecting for interactions with heterogeneous field conditions. (A) *In situ* field 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 Northen group generates repeatable

(Figure legend continued at the bottom of the next page.)

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).

Acknowledgments

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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?

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