Review

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# Challenges of modifying root traits in crops for agriculture

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**Roots play an essential role in the acquisition of water and minerals from soils. Measuring crop root architecture and assaying for changes in function can be challenging, but examples have emerged showing that modifications to roots result in higher yield and increased stress tolerance. In this review, we focus mainly on the molecular genetic advances that have been made in altering root system architecture and function in crop plants, as well as phenotyping methods. The future for the modification of crop plant roots looks promising based on recent advances, but there are also important challenges ahead.**

## Crop root architecture and function

Roots provide the interface between plants and the complex soil environment. Their key function is to mine the water and nutrients contained in soils that are required for productivity. Many factors in soils lead to spatially and temporally heterogeneous conditions, including physical properties determined by weathering and erosion, mineral nutrient content, water content, biotic factors such as soil microbial populations, and the plant communities that inhabit particular locations. The spatial heterogeneity of soils makes studying roots under field conditions a complex problem. Despite the fact that roots are hidden and require considerable effort to characterize, they are some of the most important biological tissues in our biosphere because of their unique role in extracting both water and minerals from soils that are essential for plant and animal nutrition. This review will assess the current root research landscape and the challenges that lie ahead by addressing several aspects of crop plant roots, including architectural traits, advances in phenotyping technologies, and functional traits.

## Root architectural ideotypes

Crop root architecture is determined by genetics, edaphic conditions, planting density, plant size, intercropping patterns, agronomic practices and seasonal weather patterns [1]. Although a robust plant root system is an important factor for vegetative and reproductive fitness, progress in using root system architecture (RSA) as a trait to boost crop productivity [2] has been slow.

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*Keywords:* root architecture; root phenotyping; root microbe interactions.

1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2014.08.005

Recent reviews address root ideotypes suitable for improving plant productivity by enhancing soil mineral resource capture [3–5] and by reducing root lodging [6] (Table 1). Another ideotype is disease-free roots that enable plants to forage through the soil for nutrients and water while balancing the metabolic cost of growing new roots with the cost of sustaining root function and activity. A steep–deep–cheap ideotype has been proposed to increase nitrogen and water use efficiency for crops grown under certain conditions. This model integrates root angles suitable for nitrate recovery, deep rooting for water and nutrient acquisition and a reduction of root cortical cells through the development of aerenchyma (RCA) to reduce the carbon cost of root maintenance [7]. To reduce the carbon cost of roots, another paradigm targets decreased root diameter, increased lateral root and root hair length, and increased root longevity over increased lateral root/root hair number and density [3,7]. The success of these ideotypes will be dependent on the farming systems being considered and the soil properties. For modern agriculture, high density planting is an important consideration where interplant competition may regulate root angle and root occupancy.

Root architecture ideotypes are comprised of complex, multicomponent, and interconnected traits. Root systems of various crop plants have different morphologies and these crops are cultivated under conditions with different limiting factors. Therefore, the strategies to alter architecture must be crop specific, making this a challenging task for biotechnological approaches, because a tool kit of many genes may be required. For example, corn (*Zea mays*) root systems are polymorphic and developmentally controlled (Figure 1). The post-embryonic root system consists of a primary root and variable number of branched seminal roots that predominate during the seedling stage. Other cereals such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*) have a fibrous root system [8] (Figure 2). In contrast to monocot roots, dicots such as soybean (*Glycine max*) have a taproot system with the radical developing into the primary root, from which, multiple orders of lateral roots are formed (Figure 3). Although each crop has a unique RSA, the ontogeny of some root classes is conserved across plants. For example, the embryonically derived primary root develops from the radical, whereas the postembryonic lateral, crown, brace, and basal cluster roots develop from the pericycle derived founder cells near xylem poles [9,10] or from the stem cortical parenchyma cells close to the vasculature. In many cases, genes regulating root development

**Table 1. Desired modifications in root traits that will contribute to different important ideotypes that are hypothesized or demonstrated to improve root architecture and function**

| Trait modification | Ideotype |
|---|---|
| Increased nodal root number | Root lodging resistance |
| Increased root diameter | Root lodging resistance |
| Reduced root turnover rate | Root lodging resistance |
| Seedling root vigor | Root lodging resistance and enhanced nutrient capture |
| Transporter modification | Improved root function |
| Rhizosphere pH | Improved root function |
| Enhanced root-microbe interactions | Potential for improved root function |
| Root proliferation | Enhanced nutrient capture |
| Root exudates | Enhanced nutrient capture |
| Increased lateral root number | Enhanced nutrient capture |
| Root angle and gravitropism | Steep, deep, cheap |
| Cortical aerenchyma | Steep, deep, cheap |
| Long root hairs | Steep, deep, cheap |
| Increased root hair longevity | Steep, deep, cheap |
| Increased root length density | Steep, deep, cheap |
| Long lateral roots | Steep, deep, cheap |

are conserved and may be used across multiple species, even though having a large toolbox of genes may ultimately be necessary to overcome crop specific bottlenecks in improving RSA.

## Genetics of root system architecture

Research to characterize genes and describe the genetic control of RSA has advanced in rice, corn, wheat, and soybean, but is still an area ripe for future discovery. The known genes whose genetic manipulation via overexpression or targeted suppression modifies root architecture in crops are summarized in Table 2. Additional insights can be made from the wealth of mutant studies in both corn and rice. In corn, this includes an auxin responsive LOB domain transcription factor Rootless concerning Crown and Seminal root (*RTCS*) and its downstream target Auxin Response Factor (*ARF34*), which control nodal root formation in monocots [11], the root hair mutants such as Hair Less 1 (*RTH1*), which codes for a yeast sec3 homolog, *RTH3*, which encodes a glycosylphosphatidylinositol anchor COBRA-like protein affecting root hair elongation [12], and *RTH5*, which encodes a monocot-specific NADPH oxidase [13]. The short lateral roots 1 and 2 (*slr1, slr2*) and lateral root 1 (*lrt1*) loci are reported to control lateral root development in maize; however, the underlying genes have not been cloned [14]. The *RUM1* locus encoding an Aux/IAA response regulator modulates seminal root and lateral root initiation in maize [15]. There are also multiple root architecture quantitative trait loci (QTLs) reported in maize, which control architecture and yield stability across multiple genetic backgrounds and different water regimes [16]. Other major QTLs in maize control root length, number, and dry weight, as well as root length/area co-localized with grain yield [17]. Cloning of the genes that underlie these QTLs will reveal the additional molecular mechanisms that control RSA.

Rice root architecture is controlled by many genes including: the auxin regulated Adventitious and Crown Rootless *ARL1*; *CRL1*, which encodes a LOB domain transcription factor conserved across monocots and dicots [18]; *OsCAND1*, which encodes a ubiquitin ligase homologous to AtCAND [19]; *CRL4*/*OsGNOM1*, which encodes a guanine nucleotide exchange factor [20]; *CRL5*, which encodes an AP2/ERF transcription factor [18]; the auxin biosynthetic *OsYUCC1* [21]; and the highly conserved *OsmiR393* regulatory RNA [22]. The loci regulating root elongation include *GNA1*, which encodes a glucosamine-6-P acetyltransferase, *OsCYT-INV1* (an alkaline/neutral invertase) [8,23], *Osglu3-1*(a putative membrane-bound endo-1,4-β-glucanase [24]), *OsRPK1* (a $Ca^{2+}$-independent Ser/Thr kinase [25]), and other candidates such as WUSCHEL-related homeobox 3A (*OsWOX3A*), and *OsARF16* (an integrator of phosphate starvation and auxin response [26,27]), which control lateral root development. Thus far, the majority of these genetic loci have not been targeted for transgenic manipulation, and in a few cases, the effects on RSA were accompanied by pleiotropic effects [22] (Table 2). These findings suggest the need for precision in transgene

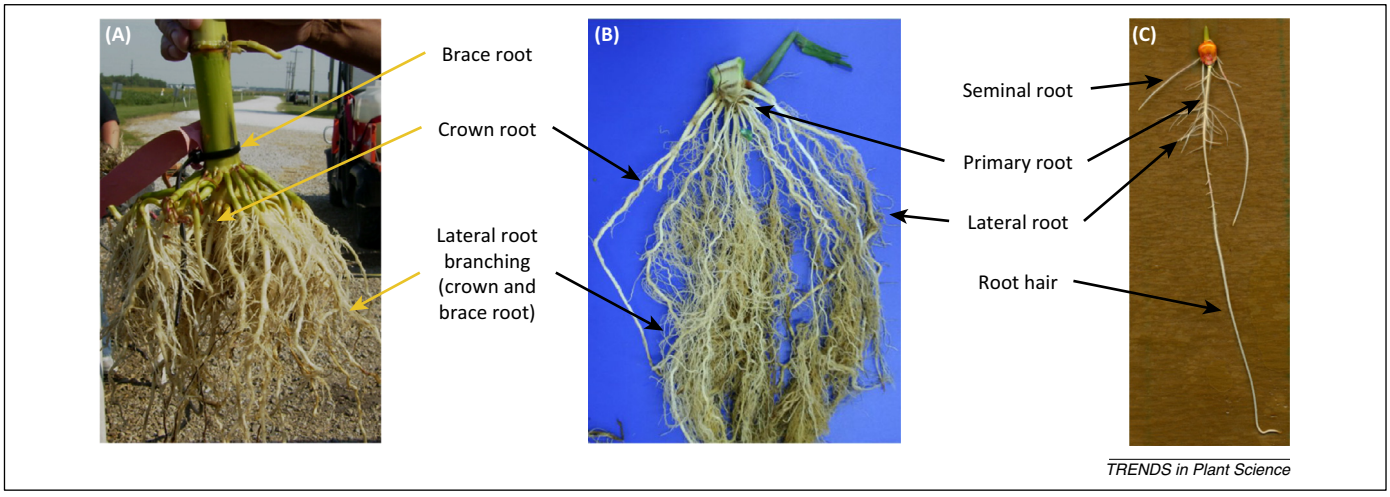


**Figure 1**. Photographs of maize root systems from **(A)** the field, **(B)** growth chamber, and **(C)** young seedlings grown on germination paper. The photos illustrate the different types of roots found in maize along with changes that occur over the course of root system development. The post-embryonic root system of corn is initially dominated by the seminal or primary root and then as the plant ages the root system becomes dominated by crown or nodal roots. (Photos courtesy of Monsanto.)

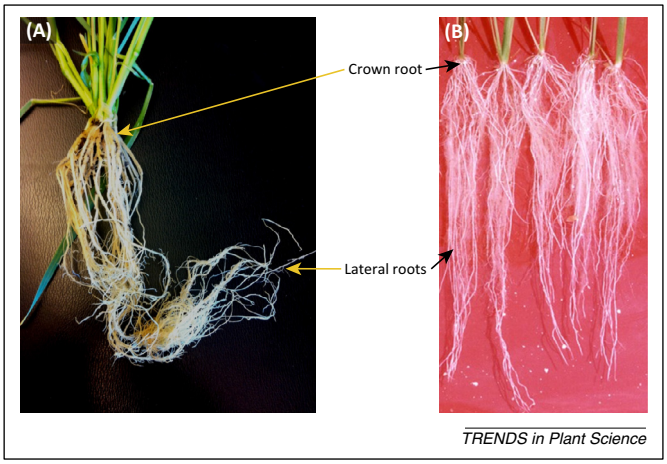


**Figure 2**. Fibrous roots are characteristic of monocots and are shown in these rice and wheat root systems. **(A)** Thirty-day-old controlled environment grown wheat plant. **(B)** Twenty-day-old controlled environment grown rice plants. Fibrous root systems contain many roots of similar size and do not have a tap root. (Photos courtesy of Monsanto.)

expression and a better understanding of genetic and functional interactions between the loci to inform gene selections.

Root hairs are also an important component of root system architecture since they increase the surface area for uptake of water and nutrients [28] and are one of the sites for plant–microbe interactions. Since root hairs are unicellular functional units of RSA, modulating root hair number and length is an alternative to improving root function depending on the soil type. The development of *Arabidopsis* root hairs (type 3 striped pattern) differs from rice root hair development due to the asymmetric cell division (type 2) [29,30]. The known root hair loci in rice share homology to *Arabidopsis* genetic counterparts. These include the auxin regulated *OsWOX3A*, which negatively regulates root hair number and length, but is a positive regulator of lateral root number (22), a putative mannosyl-oligosaccharide glucosidase (*OsMOGS*), controlling both initiation and elongation of root hair, and a homolog of *Arabidopsis GCS1/KNOPF(KNF)*, which controls seed and root epidermal cell patterning [25,31]. Genes that modulate cell wall strength and composition in root hair elongation show functional conservation between monocots and dicots, for example,

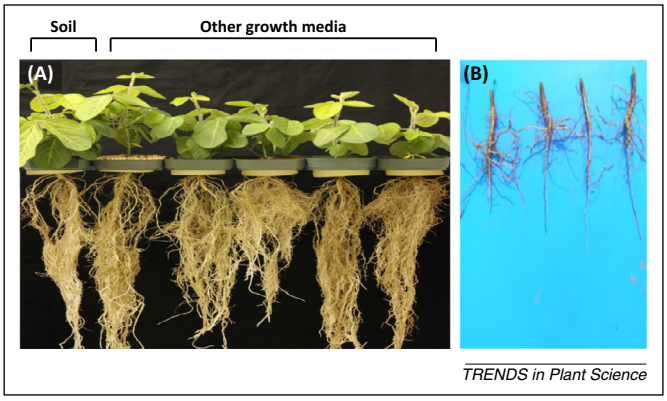


**Figure 3**. Soybean roots grown in various growth media **(A)** or grown in the field **(B)**. Dicot root systems, such as from soybean, are distinct from the characteristic monocot root systems. The soybean roots shown in this figure have a taproot system with the radical developing into the primary root from which smaller lateral roots emerge. (Photos courtesy of Monsanto.)

*OsCSLD1*, a putative functional ortholog of the *KOJAK/AtCSLD3* cellulose synthase gene [32]; *OsEXPA17*, a cell wall loosening alpha expansin whose elongation defect was complemented by *AtEXPA7* [33]; and the epidermal cell wall strength regulating xyloglucan (XyG) 6-xylosyltransferase (*OsXXT1*), which complements the root hair growth defect of the *Arabidopsis* xxt1 xxt2 double mutant [34]. Other highly conserved root hair elongation mechanisms include the PITP-mediated phospholipid signaling gene *OsSNDP1*, which is a Sec14-nodulin domain protein that complements the short root hair phenotype of the *COW1/AtSFH1* mutant [35], apyrase *RTH1*, which shares homology with *AtAPY1/2* [36] and the submergence specific regulator of root hair elongation formin homology 1 (*OsFH1*) [37], and a novel basic helix–loop–helix (bHLH) transcription factor that regulates root hair elongation (*OsRHL1*) whose *Arabidopsis* functional counterpart is yet to be discovered [38]. With the exception of *OsEXPA8* [39], there are no reports in rice where root hair traits are subject to transgenic manipulation. Conservation of gene function between rice and *Arabidopsis* offers the possibility to quickly identify genes involved in cell extension using a model system that can then be applied to a crop plant. Since the ontogeny of root hairs varies between the two species, know-how regarding how cell fate is specified in rice will shed light on the ways to achieve epidermal cell type independent root hair differentiation, and whether it benefits rice productivity.

The genetic basis of dicot root system architecture has been studied extensively in *Arabidopsis thaliana*, which serves as an important resource for understanding the molecular genetic control of tap root systems. In contrast to *Arabidopsis*, the characterization of genes or the genetic loci controlling RSA in soybean is limited. Recently, five QTLs from soy recombinant inbred lines were found to explain 7–15% of the phenotypic variation in fibrous root scores [40]. These are potential future targets for the introduction of root traits into elite cultivars for improving soybean yield under drought stress. The use of QTLs to manipulate root architecture will be difficult until markers are developed or until the gene(s) underlying the trait is cloned.

### *Genetic engineering to modify RSA in crops*

Despite some gaps in our understanding of the genetic mechanisms controlling RSA in crop plants, several studies report success in modifying crop RSA traits (Table 2) using different types of promoters and phenotyping methods. The most progress has been reported in rice including overexpression of transcription factors *OsNAC5/9* and *OsMYB2*, the receptor kinase *PSTOL1*, the G-protein coding Root Architecture Associated (*OsRAA1*), a cell wall extension *OsEXPA8* gene and the identification of the *DRO1* allele (Table 2).

In some cases genes that alter RSA also increase phosphorus, nitrogen, and water use efficiency, leading to higher grain yield, indicating that root traits are important for increasing yield. Two outstanding examples of where genes have been identified that confer changes in root architecture are *DRO1* [41] and *PSTOL1* [42]. Growing longer, deeper roots is one approach to increasing water uptake when it is available deeper in the soil profile. *DRO1* is an example in

**Table 2. Summary of gene targets to modify RSA in crop plants**

| Crop | Approach | Gene (constitutive promoter used except where noted) | Phenotype | Development stage of root phenotyping | Growth method | Refs |
|---|---|---|---|---|---|---|
| Soybean | Over-expression | *GmWNK1* WNK protein kinase | Small plants; reduced lateral root number and length | Early reproductive (35 days old) | Hydroponics | [85] |
| Soybean | Composite plants using hairy roots | *GmEXPB2* β-expansin | Phosphorus use efficiency and increased root length | Soybean hairy roots | *In vitro* root culture | [86] |
| Corn | Over-expression | *LOS5*, MoCo sulfurase | Increased root biomass under water stress, reduced wilting | Early vegetative (28–45 days old) | Pot study using vermiculite and sand | [87] |
| Corn | Over-expression | *ZOG1*, zeatin O-glucosylation | Increased root mass and branching, smaller plant, reduced seed weight | Early vegetative 21 days old | Information not found in paper | [88] |
| Rice | Over-expression | *OsMYB4P R2R3* MYB transcription factor | Increased primary length, lateral root length and density, shoot biomass, phosphorus use efficiency | Early vegetative (~14 days old) | *In vitro* | [89] |
| Rice | Over-expression | Root promoter *OsCKX4* cytokinin oxidase | Increased crown root length, number, dry weight, shoot size unaltered | 21-day-old seedling | *In vitro* | [90] |
| Rice | Over-expression | OsEXPA8 α-expansin | Increased plant height, leaf number and size. Increased primary root length, lateral roots, root hairs | Mature plants (60 days old) 7-day-old seedling for root hair evaluation | Pot study for RSA; gel based for root hair | [39] |
| Rice | Over-expression | *OsHsf7* Heat shock | Salinity and drought tolerance, longer primary root and adventitious roots, fewer shorter lateral and root hairs | Seedling (5, 7, 15 days old) | Pot study for stress | [91] |
| Rice | Over-expression | *OsWOX11* WUSCHEL-related homeobox | Precocious crown root growth | 14-day-old seedling | Pot study | [92] |
| Rice | Downregulation | *OsWOX11* WUSCHEL-related homeobox | Short primary root, fewer crown roots, reduced plant height | 14-day-old seedling | Pot study | [92] |
| Rice | Allele specific expression | *DRO1* unknown function | Decreased root angle, drought tolerance and increased yield under drought stress | Seedling | Gel; field based root trench | [93] |
| Rice | Over-expression | *1* Stearoyl-acyl carrier fatty acid desaturase protein family | Longer lateral roots | Seedling (15 days after germination) | Hydroponics | [94] |
| Rice | Over-expression | Root specific promoter *OsNAC9* transcription factor | Increased grain yield; drought tolerance increased root diameter, aerenchyma | Before heading stage | Pot study with soil | [95] |
| Rice | Over-expression | *OsNAC9* transcriptional regulator | Increased grain yield increased drought tolerance; increased root length, volume, root dry weight, diameter, stele | Before heading stage | Pot study with soil | [95] |
| Rice | Over-expression | Root specific promoter *OsNAC5* transcriptional regulator | Increased grain yield under stress and non-stress; larger roots; increased cortex and stele | Before heading stage | Pot study with soil | [96] |
| Rice | Over-expression | *OsNAC5* transcriptional regulator | Increased yield under non-stress; larger roots; increased cortex and stele | Before heading stage | Pot study with soil | [96] |
| Rice | Over-expression | *miR393a TIR1* homolog | Long primary root, reduced crown root, reduced plant height, leaf angle, reduced seed size | 28-day-old plants | Hydroponics | [22] |
| Rice | Over-expression | *OsMYB2P-1* transcriptional regulator | Increased primary and adventitious root length, root-shoot dry weight, plant height under low phosphorus | Early vegetative, 21-day-old plants | Hydroponics | [97] |

**Table 2 (*Continued*)**

| Crop | Approach | Gene (constitutive promoter used except where noted) | Phenotype | Development stage of root phenotyping | Growth method | Refs |
|---|---|---|---|---|---|---|
| Rice | Over-expression | *OsSPIKE/NAL1* Auxin transport | Increased total spikelet number per panicle, root dry weight | Maturity | Field | [98] |
| Rice | RNA interference | Alternatively spliced, CCT domain protein nutrition response and root growth (NRR) | Increased root length under nitrogen and phosphorus stress | Early vegetative, 12-day-old seedlings | Hydroponics | [99] |
| Rice | Over-expression | *OsPSTOL1* receptor-like kinases | Higher root dry weight; larger root system; increased total root length and root surface area; enhanced nutrient uptake (nitrogen, potassium), grain weight, increased phosphorus use efficiency | Early vegetative; 21 days after germination (DAG) | Hydroponics; pot study | [42] |
| Rice | Over-expression | *SiPf40* Zinc or iron transporter protein | Lateral root development; changes in hormone levels; increased tillering | Information not found in paper | Information not found in paper | [100] |
| Rice | Over-expression | Root epidermal promoter *AlaAT* Alanine amino transferase | Increased root biomass; yield; NUE | 45-day-old plant | Hydroponics | [101] |
| Rice | Over-expression | *OsRAA1G* Protein | Primary root inhibition and lateral root proliferation; delayed gravitropic response long leaves; seed sterile | Early vegetative 12-day-old seedlings | Information not found in paper | [102] |
| Rice | Over-expression | *AtFPF1* Flower promoting factor of unknown function | Primary root inhibition and adventitious root proliferation | 12 days after germination; maturity | Pot study | [103] |
| Rice | Antisense | *OsYUCCA1* | Dwarf shoot, short root | Regenerating calli | *In vitro* | [21] |
| Rice | Mutant | Rice leaf tip necrosis 1 (*LTN1*), ubiquitin-conjugating domain protein | Longer primary and adventitious roots under inorganic phosphorus starvation | 10-day-old seedling | Hydroponics | [104] |
| Barley | RNA interference | Cytokinin oxidase | Increased yield; root weight and length | 5-day-old seedling | Information not found in paper | [105,106] |
| Wheat | Over-expression | *GmbZIP1* | Drought tolerance; increased root and shoot growth | 3-week-old plants | Gel | [107] |

rice where root angle and rooting depth [41] can now be targeted by breeding or using transgenic approaches to achieve the steep–deep ideotype [7]. In rice, the *DRO1* allele was identified from Kinandang Patongan, which is an upland cultivar. Introgression of this allele using traditional breeding and expression of the *DRO1* genomic fragment using transgenic approaches in IR64 provides evidence that steep–deep root architecture increases yield under drought conditions. *PSTOL1* encodes a receptor-like kinase that maps to a major QTL for phosphorus deficiency tolerance in rice [42] and has been shown to increase root biomass. Transcriptional analysis of *PSTOL1* overexpression lines led to the identification of 23 differentially regulated genes functionally related to root growth and stress responses. Identification of these loci highlights the importance of root growth and angle as traits that impact tolerance to drought and phosphorus deficiency.

Another example of molecular genetic manipulation of RSA is the overexpression of cytokinin dehydrogenase *AtCKX3*, which catalyzes the irreversible degradation of cytokinins. When *AtCKX3* was expressed under a root specific promoter, this resulted in increased root meristem size and root biomass in *Arabidopsis* [43]. Constitutive expression of the same gene under the 35S promoter led to reduced shoot growth and increased root growth. Similar to results in *Arabidopsis*, the constitutive overexpression of *HvCKX1* or *HvCKX9* in barley resulted in increased root growth, but reduced shoot growth and infertility [44]. The reduced shoot growth and an infertility phenotype were abrogated by using a root targeted promoter [44]. The results highlight the important role of tissue-dependent phytohormone homeostasis in controlling organ size and reducing undesirable phenotypes caused by constitutive promoters.

## Root phenotyping

Root system architectural variation in crop species is likely to influence crop performance [7,45]. Propelled by technical advances in model species, such as *Arabidopsis*, a growing number of crop root system architecture phenotyping platforms have been recently developed, or enhanced (Table 3). Neuman *et al.* [46] provides a very detailed review of root rhizosphere assays and methodologies, whereas only a brief updated overview is presented in this review. The

**Table 3. Summary of studies that have used different root phenotyping platforms**

| Crop | *In situ* vs *ex situ* | Phenotyping platform[a] | Growth stage | Image analysis | Refs |
|---|---|---|---|---|---|
| Corn | *In situ* | CE – rhizotron | Vegetative | Yes | [108] |
| Corn | *In situ* | CE – soil | Vegetative | Yes | [59] |
| Corn | *In situ* | Field – rhizotron | Reproductive | Yes | [109] |
| Corn | *Ex situ* | CE – aeroponics | Early vegetative | Yes | [110] |
| Corn | *Ex situ* | CE – growth pouch | Early vegetative | No | [111] |
| Corn | *Ex situ* | CE – growth pouch | Early vegetative | Yes | [112] |
| Corn | *Ex situ* | CE – hydroponics | Vegetative | No | [113] |
| Corn | *Ex situ* | CE – sand | Vegetative | Yes | [114] |
| Corn | *Ex situ* | Field – shovel | Reproductive | No | [48] |
| Corn, barley, brassica, rice | *In situ* | CE – rhizotron | Reproductive | Yes | [55] |
| Corn, rice | *Ex situ* | CE – hydroponics | Early vegetative | Yes | [45] |
| Corn, sorghum | *In situ* | CE – rhizotron | Vegetative | Yes | [115] |
| Corn, wheat | *Ex situ* | CE – soil | Early vegetative | Yes | [57] |
| Rice | *In situ* | CE – transparent media | Early vegetative | Yes | [53] |
| Rice | *In situ* | CE – transparent media | Vegetative | Yes | [47] |
| Rice | *Ex situ* | CE – soil | Reproductive | Yes | [116] |
| Rice, soybean | *In situ* | CE – transparent media | Early vegetative | Yes | [1] |
| Soybean | *Ex situ* | CE – hydroponics | Early vegetative | No | [52] |
| Wheat | *In situ* | CE – transparent media | Early vegetative | No | [117] |
| Wheat | *Ex situ* | CE – soil | Vegetative | Yes | [56] |
| Wheat | *Ex situ* | Field – soil core | Reproductive | Yes | [49] |
| Wheat, barley | *In situ* | Field – soil | Reproductive | No | [60] |

[a]Abbreviation: CE, controlled environment.

ability to easily, accurately, and extensively characterize root phenotypes is the major challenge in the field of root biology.

Root phenotyping platforms can be defined by the combination of growing environment and rooting media and broadly grouped into *ex situ* or *in situ* methodologies. Methodologies can also be described as static (single time point) and dynamic (rate changes over time) metrics across both local (individual roots) and global (root system architecture) regions of the roots [45]. Examples of local root metrics include root length, root branching, and root diameter. Each of these can be described in static or dynamic observations [1,45,47]. Root surface area, root density, and root volume are examples of global root metrics. *Ex situ* platforms require the roots to be extracted from the medium prior to root characterization and, therefore, capture only a static assessment of root architecture metrics. *In situ* platforms enable direct imaging of roots within the growth medium and have created opportunities to characterize dynamic metrics.

*Ex situ* platforms enable relatively rapid assessment of static root metrics and have the potential to be implemented across a wide range of developmental stages. *Ex situ* field platforms developed for both corn [48] and wheat [49] provide the characterization of local root metrics, with some inclusion of image-based analyses, maintain flexibility in developmental timing of analyses, but significantly increase the time required for digging, washing, and subsequent analysis. Controlled environment platforms that are complementary to these field platforms include solid media, for example, soil, sand, or Turface, or liquid media such as hydroponic, aeroponic, or germination paper [45,49–52]. Controlled environment platforms are often limited to vegetative stages, but provide detailed characterization of root metrics through image analyses and are easier for the application of stress treatments. The use of appropriate platforms for root phenotyping can provide valuable information for the characterization of phenotypic variation in germplasm and transgenic experiments.

Recently developed *in situ* platforms expand the ability to assess dynamic root metrics and have increased the potential to describe global root system architecture. Transparent growth media, such as gellan gum or phytagel, [1,47,53,54] are each unique platforms that utilize 2D imaging technology or 3D root scanning to recreate global root system architecture. In each of these platforms, repeated imaging up to 18 days postgermination delivers dynamic root metrics. Furthermore, novel global metrics, such as circumnutation or gravitropism can be derived from the images [47,53]. By contrast with the relatively labor intensive transparent media platforms, an *in situ* automated controlled environment soil-based platform was developed for characterization of multiple crops (maize, rice, and brassica [55]). Although this rhizotron does not provide some of the global metrics that are available in the transparent media platform, it is superior in its ability to provide data during later developmental stages, and greatly decreases labor costs required for digging and washing roots. Novel *in situ* platforms utilizing X-ray tomography [56,57], nuclear magnetic resonance imaging [58,59], or electrical capacitance [60] are in development, but have not been implemented in broad screening experiments due to low throughput and expense of the instrumentation. The technical precision and comprehensive phenotypic analyses of the *in situ* platforms exceed the *ex situ* platforms, but are often more expensive and have a greatly reduced throughput.

Current root system architecture platforms do not include the collection of root cellular morphology data such as tissue or cellular anatomy. Although the measurement of cellular morphology is relatively time intensive due to the sample preparation required for microscopy, significant variation in root anatomy metrics has been observed in root cortical aerenchyma, root vasculature, and root hairs [50,61,62]. Variation in root cortical aerenchyma has been suggested as a potentially important trait [7] because it may lower the carbon cost of maintaining active roots. Therefore, future advances in the ability to collect root system architecture information, including root anatomical descriptions, has the potential to contribute to the development of new root traits that will increase crop productivity. Although cheaper roots [7] are an attractive concept, it will be important to ensure that root strength and the ability to grow through regions of compacted soils or high bulk density is maintained. This and other trade-offs with the ideotype of steep–deep–cheap traits have been considered and tested in some plant species [63,64]

## Root function – water

Water uptake by roots is essential for plant growth and for high yields, but there have only been a few approaches explored to modify the water uptake capacity of roots. In certain climates, saving water for later stages of development is important for increasing yield of crops, such as wheat [65]. To reduce water uptake and save soil water for later stages of development xylem vessel diameter was genetically reduced based on the hypothesis that this would reduce the root hydraulic conductance. Although water use was not reported, yield did increase between 3 and 11% under dry conditions across years in wheat backcross lines that had been selected for more narrow xylem diameter [65], but the observed yield differences were not significant.

Modification of aquaporin (AQP) expression is another avenue for changing root water uptake. In an early study with *Arabidopsis*, the knockout of a single AQP (*PIP2.2*) resulted in a reduction in hydraulic conductivity (Lp) of the roots by 25–30% [66]. In *Arabidopsis*, variation in root hydraulic conductivity was explored in 13 naturally occurring accessions. While results suggested a link between Lp and water channel gene expression, factors other than water channel expression, such as environmental conditions (salinity), also interact strongly with hydraulic conductivity [67]. In corn, the expression of several AQPs has been correlated to diurnal changes in root hydraulic conductivity [68], suggesting that in at least one crop plant, the manipulation of water channels may be used to modify the water uptake function of roots. Changes in root water uptake characteristics using anatomical and functional approaches, such as the modulation of water channels, could be more intensively explored in crop plants as an approach for saving water in drought prone regions.

## Root function – nutrients

Increasing nutrient uptake efficiency from soils is a major challenge because of the wide range of soil types in which crops are cultivated, but is very important because of the contribution that the additional extraction of nutrients could make to reducing stress and increasing the efficiency of nutrient capture. Attempts to increase root uptake of nitrogen in rice has been approached through the overexpression of the AMT type ammonium transporters. Two studies on the overexpression of *OsAMT1.1* in rice showed that uptake of ammonium could be altered following nitrogen deprivation [69], and also under high nitrogen conditions in one, but not another, rice cultivar [70]. Although there were differences in the results of these two studies, in part based on the two genotypes that were used, the overall effects of increased ammonium uptake did not result in enhanced growth of rice. In fact, in one study [69], the increased uptake led to a decrease in biomass.

Phosphate transporter overexpression has been tested as one approach to increase phosphorus uptake. Early studies showed that overexpression of a phosphate transporter increased phosphorus uptake in suspension cells [71], but it did not increase phosphorus uptake in whole barley plants [72]. This may be due to the promoter used or factors related to the regulation of the transporter. In another report [73] *OsPT1* expressed using a constitutive promoter in rice increased tissue concentrations of phosphorus over than wild type, but plants were shorter and had more tillers than wild type plants. Another phosphate transporter *OsPT1.8* was overexpressed in rice, and tissue concentrations of phosphorus increased when plants were grown on high concentrations of phosphorus, but not when concentrations were below 40 mg inorganic phosphorus/kg soil [74].

Since phosphorus availability is often limiting uptake, one promising area of research for improving phosphorus uptake is to increase availability through the overexpression and increased release of phosphatases from roots [75]. One of the several examples of this strategy is *AtPAP15* [76], which was overexpressed in both *Arabidopsis* and soybean using a constitutive promoter. A 1.5–2.6-fold increase in phytase activity was secreted from roots which led to increased dry weight and phosphorus content when plants were grown in sand culture where phytate was the sole phosphorus source [76]. In other examples where this approach has been tried in the field, where substrate concentrations are limiting, there was no improvement in plant phosphorus nutrition [77]. Therefore, increased exudation of a phosphatase may only be useful under certain field conditions where inorganic phosphorus is low, but organic phosphorus that plants would not normally be able to access, such as phytate, is present. More large scale field testing in different soil types will be required to fully assess the potential for this approach.

In many soils, phosphorus is not available due to physicochemical constraints that limit solubility of this essential mineral nutrient. In one study, the ectopic expression of a malate transporter increased the ability of barley to acquire phosphorus on acid soils, which led to higher internal phosphorus concentrations and increased yield [78]. Increasing uptake of nutrients through the overexpression of nutrient transporters is obviously a challenge and will not be appropriate in all situations. More work is needed using creative approaches, such as combining several genes to engineer pathways, or multiple steps in a physiological process, such as enhanced malate exudation together with increased transporter expression.

Similar to phosphorus, iron is often present in soils, but is unavailable for uptake by roots. Dicots and monocots have evolved different strategies to increase the availability of iron, which could be used to improve crop plant performance. Cereals have specialized mechanisms for increasing the availability of iron through the secretion of chelating compounds called phytosiderophores. Using microarrays and bioinformatic approaches, the transporters that control the secretion of the phytosiderophores nicotianamine and deoxymugineic acid were identified and characterized to demonstrate their function and role in iron acquisition [79]. In dicot crop plants, and also in rice, a reduction strategy is used to increase the availability of iron by converting the unavailable form $Fe^{3+}$ to $Fe^{2+}$. In rice and soybean, overexpression of an $Fe^{3+}$ reductase led to increased tolerance to low iron conditions [80,81] suggesting that transgenic manipulation of these mechanisms holds promise for increasing yields where iron availability is low.

The traits and genes controlling tolerance to acid soils is an outstanding example of where root function can be modified with a transgene. In wheat, the amount of malate effluxed from root apices was correlated with the relative acid tolerance of multiple genotypes [82], and the gene underlying the malate efflux trait was cloned through a subtractive hybridization of cDNAs [83]. In cereals such as corn, sorghum, and barley, a similar mechanism of organic acid exudation has been shown to confer aluminum tolerance, with citrate being the predominant organic acid secreted by roots by a member of the multidrug and toxic compound extrusion (MATE) family in corn [84]. These genes can now be engineered into other genotypes of wheat, corn, sorghum, and barley to improve yields on acid soils.

It has been very exciting to see the progress that has been made over the past 10 years in modifying root function. Modification of root function should continue to be a productive area for future applications to agriculture, and may eventually enhance yields of crop plants in soils that have optimal amounts of nutrients. To ensure success in this area, we will need to more fully understand how to best regulate the expression of transporters to create more nutrient use-efficient crops that do not have any yield penalty.

## Concluding remarks

The study of roots has advanced to the point where modifications can be made to both architecture and function using molecular tools. Although there are several root ideotypes predicted to be suitable for improving crop productivity, a challenge will be to find the winning combination of shoot and root traits that can be successfully combined to benefit whole plant growth and productivity. However, a major challenge is our ability to easily phenotype roots, particularly under field conditions. The development of non-invasive and high-throughput phenotyping methods is needed to measure the changes in root architecture and function in field.

Although not discussed in this review, the interaction between crop roots and rhizosphere microbes also holds promise as a future approach to potentially increasing plant nutrient uptake, enhancing drought tolerance, and ultimately improving yield (http://academy.asm.org/index.php/browse-all-reports/800-how-microbes-can-help-feed-the-world). Research in this area will need to focus on discovery of novel microbes and a deep understanding of how plant roots recruit or enrich for microbes in and around the roots. Past success indicates that the modification of root structure and function will be a productive approach to increasing crop yield, with the next big challenge being to understand root soil microbe interactions, and to manipulate these interactions to provide benefits for plants.

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