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Uncovering the hidden half of plants using new advances in root phenotyping

Jonathan A Atkinson[1], Michael P Pound[1,2], Malcolm J Bennett[1] and Darren M Wells[1]

Major increases in crop yield are required to keep pace with population growth and climate change. Improvements to the architecture of crop roots promise to deliver increases in water and nutrient use efficiency but profiling the root phenome (i.e. its structure and function) represents a major bottleneck. We describe how advances in imaging and sensor technologies are making root phenomic studies possible. However, methodological advances in acquisition, handling and processing of the resulting 'big-data' is becoming increasingly important. Advances in automated image analysis approaches such as Deep Learning promise to transform the root phenotyping landscape. Collectively, these innovations are helping drive the selection of the next-generation of crops to deliver real world impact for ongoing global food security efforts.

Addresses
[1] School of Biosciences, University of Nottingham, Sutton Bonington, UK
[2] School of Computer Science, University of Nottingham, Nottingham, UK

Corresponding authors: Bennett, Malcolm J (malcolm.bennett@nottingham.ac.uk),
Wells, Darren M (darren.wells@nottingham.ac.uk)

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Introduction

Crop production has to double by 2050 to keep pace with global population growth. This target is even more challenging given the impact of climate change on water availability and the drive to reduce fertilizer inputs to make agriculture environmentally sustainable. Developing crops with improved water and nutrient uptake efficiency would provide a solution. As root architecture influences nutrient and water uptake efficiency, a 'second green revolution' has been proposed that deploys crops with improved below ground traits [1]. However, selecting crops based on root system architecture (RSA) poses practical challenges.

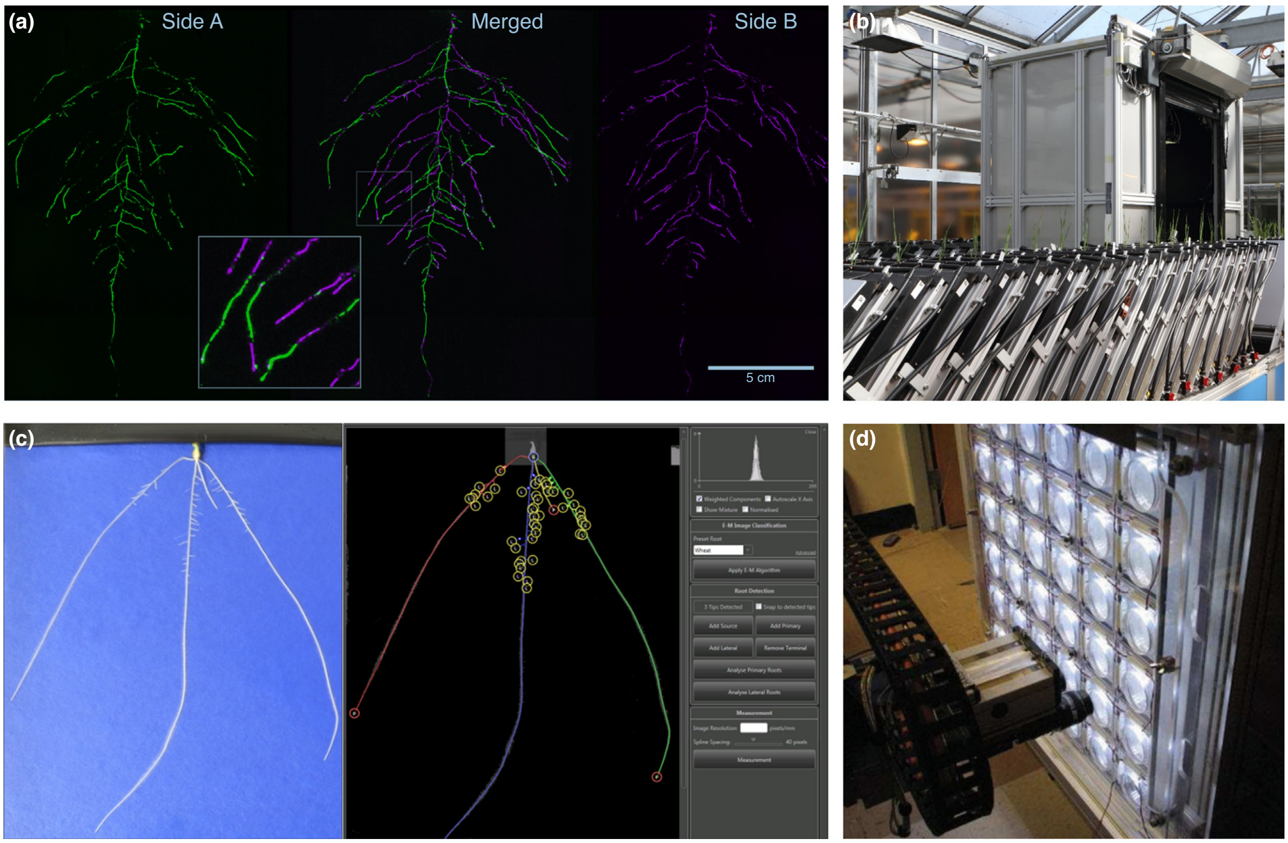
This review discusses recent advances in root phenotyping. To date, classical non-destructive 2D techniques such as agar plates or rhizotrons have been integral to our understanding of root development (Figure 1). Non-destructive analysis of 3D root growth is also possible using transparent gels [2,3•,4] but results are often difficult to extrapolate to field conditions. To non-invasively study 3D root growth in soil, more sophisticated approaches are needed. This review explores several promising new approaches to uncover the 'hidden half' of plants grown under either lab or field conditions (Box 1). We discuss the 'big data' challenges associated with root phenotyping and describe promising solutions being developed by other disciplines, then conclude with a forward look.

Technologies for root phenotyping under controlled conditions

The opaque nature of soil makes phenotyping root systems *in situ* challenging compared to analysing above-ground plant organs. Non-destructive techniques under controlled conditions have traditionally relied on rhizotrons (enclosures with transparent or removable observation windows), growth pouches, or transparent artificial growth media (Figure 1). Images are usually two-dimensional (2D) and, if soil is used, often fail to capture the complete root system architecture as many roots will be occluded by soil particles. The GLO-Root system designed for *Arabidopsis* [5••] mitigates these effects by using luminescence-based reporters for visualisation of architecture and gene expression patterns, and by combining images from both sides of the rhizotron (Figure 1). Soil-free techniques such as hydroponics, aeroponics, gel plates, and growth pouches provide greater contrast between root and substrate allowing accurate extraction of root system architecture, although the root systems of plants grown in artificial media can vary considerably from those grown in soil [6]. Pouch systems using plants grown vertically on germination paper have been successfully used in seedling screens for many species including, bean [7], maize [8], wheat [9], oilseed rape [10], and pearl millet [11]. Despite their limitations, 2D soil and artificial media systems are widely used due to their suitability for incorporation into high-throughput root phenotyping platforms such as GrowScreen-Rhizo [12], Phytomorph [13], GrowScreen-PaGe [10], RADIX [14] and RhizoTubes [15].

Plant root systems are three-dimensional (3D) structures with many features that are difficult to quantify in 2D [3•]

Figure 1



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2D imaging of plant roots. **(a)** GLO-Roots [5••]. *Arabidopsis* plant expressing a luminescent reporter imaged on each side of the rhizotron (coloured green and magenta respectively) at 21 days after sowing (DAS). **(b)** GROWSCREEN-Rhizo [12]. A high-throughput automated root phenotyping platform using soil-filled rhizotrons. **(c)** Pouch system [9] for cereal seedlings (left panel). *RootNav* [51] analysis software (right panel). **(d)** Phytomorph [13] A high-throughput robotic imaging platform for *Arabidopsis* growing on agar plates.

such as the arrangement of seminal roots at the root crown of cereals (that are often asymmetrically distributed), and the angle and number of roots and root whorls in maize crowns. Dynamic growth responses such as gravitropism and circumnutation are also more readily studied in 3D [2]. Three-dimensional representations of root systems can be produced from multiple-viewpoint imaging of plants grown in optically transparent media [2,3•,4] or hydroponically using a support system [16]. One such system was successfully used to uncover the underlying genetic basis for several 3D root architectural traits in rice not revealed by 2D phenotyping [3•]. Non-destructive, 3D phenotyping of roots in soil is currently achievable using three tomographic techniques originally developed for medical applications (Figure 2): X-ray computed tomography (X-ray CT), magnetic resonance imaging (MRI) and positron emission tomography (PET).

X-ray CT allows the visualisation of 3D volumes based on differential X-ray attenuation. Although first demonstrated in plant roots over 30 years ago [17], only recently has advances in scan time, resolution, reconstruction times, and image segmentation software made X-ray CT a viable technology for root phenotyping in soil [18]. X-ray CT has been used to examine the cultivar-specific response of rice root systems to growth medium texture [19]; patterning of lateral roots in *Arabidopsis*, maize, and rice [20]; inter-specific interactions between aspen and spruce [21]; and to quantify roots of prairie dropseed to parameterise computational fluid dynamics simulations [22]. MRI employs radio-frequency waves and strong magnetic fields to stimulate atoms (usually of hydrogen in water) and produce a 3D spatial map [23••]. MRI has been employed to image the root systems of soil-grown maize, bean, sugar beet, and barley [23••,24,25]. PET scanning visualizes the distribution of short half-life radioactive tracers, such as carbon isotopes used in plant metabolic processes [26]. Despite a high sensitivity for tracers, PET is currently limited to a relatively coarse resolution of ~1.4 mm [24]. To overcome this limitation,

Box 1 Glossary of technologies applied to plant root phenotyping

Electrical resistance tomography (ERT) — imaging of sub-surface soil structure from maps of electrical resistivity measured via buried probes.

Electromagnetic inductance (EMI) — mapping of spatial soil electrical conductivity using sensors held above the soil surface.

Ground penetrating radar (GPR) — mapping of sub-surface structure by measuring reflection, refraction, and scattering of pulses of high-frequency radio waves. Receiving antennae may be positioned in contact with the soil or above the soil surface.

Magnetic resonance imaging (MRI) — imaging technique based on absorption and re-emission of electromagnetic radiation from nuclei in a magnetic field.

Positron emission tomography (PET) — imaging technique based on detection of gamma radiation from tracer molecules.

Rhizotron — a growth chamber with transparent or removable observation windows through which roots can be imaged.

X-ray computed tomography (X-ray CT) — imaging technique based on attenuation of X-rays to create cross-sections for reconstruction into a 3D model.

PET is often combined with other tomographic techniques including MRI [24], X-ray CT [26], and optical projection tomography [4].

Both X-ray CT and MRI can be used for monitoring root system growth over time, with no adverse effects of repeated scanning on plant development [23••,27], although care should be taken in X-ray CT experiments to monitor any impact of repeated scans on root development and soil biota [27]. MRI and X-ray CT can be seen as complimentary technologies, with their own advantages and limitations [28]. However, MRI is more dependent than X-ray CT on the choice of substrate with initial experiments requiring specific soils or the removal of ferromagnetic particles [29]. A recent study of eight substrates (6 natural soils and 2 artificial mixtures) reported five suitable for root segmentation via MRI in barley plants (including the two artificial mixtures). Of the three natural soils deemed unsuitable to MRI, two were able to be used to resolve thicker roots [29]. Both MRI and X-ray CT imaging are confounded by high soil moisture, although both techniques are suitable for soils held at field capacity or lower [29,30]. In their medical applications, CT scanners and MRI machines are usually arranged for horizontal sample loading. The availability of non-medical X-ray CT scanners with vertical sample loading has, combined with the lower equipment cost, led to a wider adoption of this technology for plant phenotyping compared to MRI.

Root phenotyping in the field

Field-based phenotyping has seen significant advances in recent years with sensor technologies to quantify canopy traits (including LIDAR, multi- and hyperspectral imaging, thermography, and RGB imaging) deployed on drones, tractor mounts and gantry systems [31–35]. Phenotyping for root traits in the field has seen comparatively less advancement, largely due to the difficulties associated with imaging below-ground. Classic methods such as the soil core-break are widely used, with this protocol being recently improved by employing UV illumination and fluorescence spectroscopy to enhance soil-root image contrast and allowing automated image capture and processing of core-break faces [36].

Shovelomics [37], or root crown phenotyping, is one of the most widely adopted high-throughput field methods. The protocol, originally designed for maize, has been adapted for other species including legumes [38] and wheat [39]. Shovelomics generates a number of key root architecture parameters including crown root number and angle [37]. Automatic image analysis software such as DIRT [40] and REST [41] have further increased throughput; however, the rate-limiting step is still the manual excavation of the crown root system. Automation of this labour-intensive process is being addressed by researchers at Pennsylvania State University in the DEEPER project, part of the ARPA-E funded ROOTS program that includes *inter alia* development of field-deployable X-ray CT and MRI platforms [42].

Development of non-invasive geophysical techniques to study the root and soil profile has also advanced in recent years. This includes Electrical Resistance Tomography (ERT) that measures soil water profiles. Most commonly used to analyse large diameter root profiles (e.g. trees [43]), ERT has seen limited adoption to date in the areas of crop phenotyping [44]. Although ERT is non-destructive and significantly higher throughput than traditional coring methods, it is limited by the number of probe arrays that can be placed in the field and is still considered low throughput compared to other geophysical approaches such as electromagnetic inductance (EMI). EMI is significantly higher throughput than ERT as it does not require probes or direct contact with the soil [45] and was recently implemented to quantify root activity in wheat [46••]. For a detailed comparison of ERT, EMI and penetrometer methods for measuring differences in soil water profiles at the plot scale between genotypes, see [46••].

Ground penetrating radar (GPR), another geophysical technique of similar throughput to EMI, uses high frequency radio waves to detect objects or boundaries between materials in the ground based on their permittivity. GPR, like EMI, has been used to detect and quantify tree roots, but does not currently have the resolution to detect roots less than 2 mm in diameter (reviewed in [47]). Despite this, GPR has recently been used to detect bulk root biomass in wheat and sugar cane (although with limited ability to detect differences between genotypes [48]) and shows potential as a future

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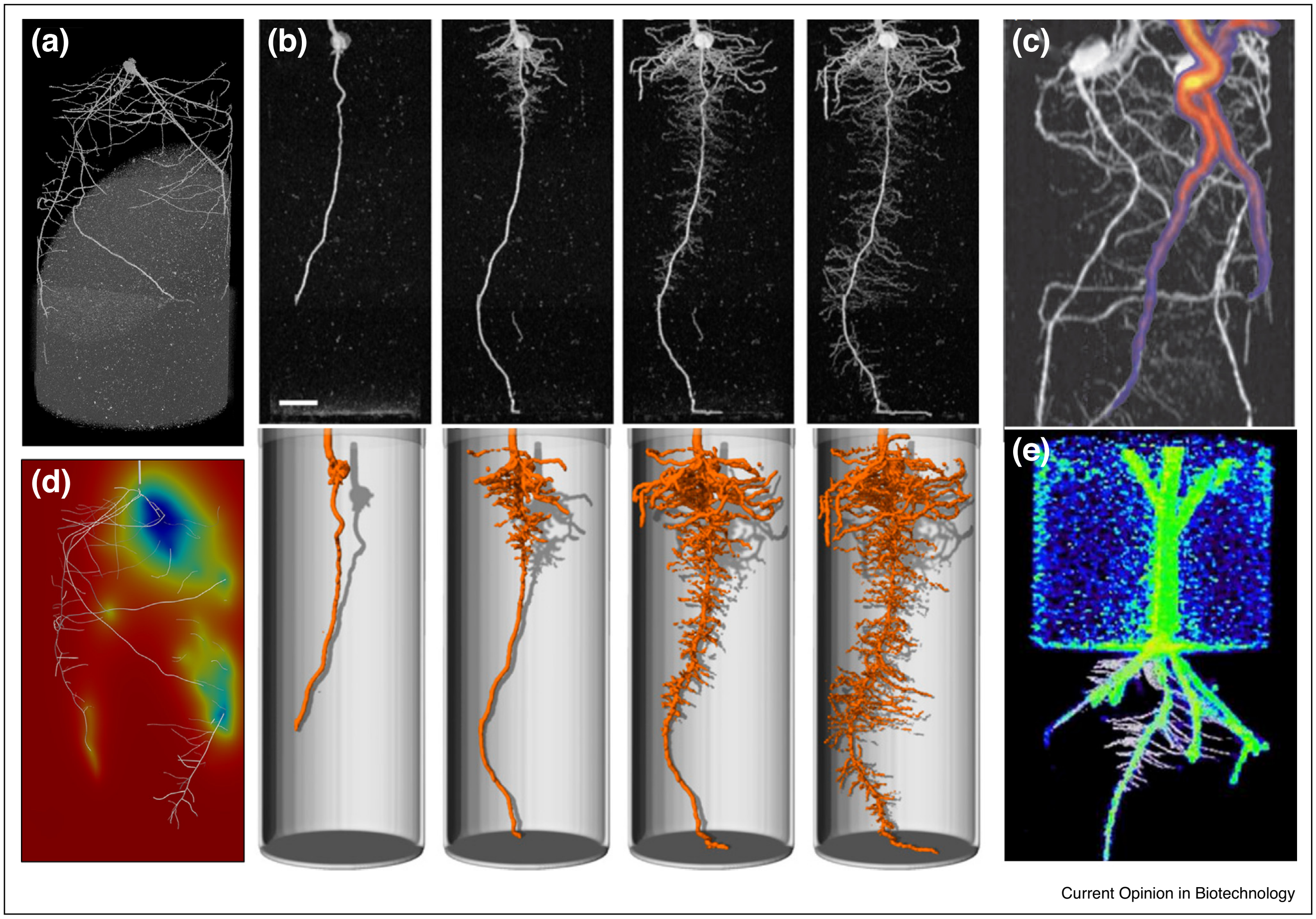
Figure 2



3D tomographic imaging of plant roots. **(a)** X-ray CT micrograph of a wheat seedling 12 DAS. **(b)** MRI imaging of a maize root system at 6, 9, 12, and 15 DAS [23••]. Upper panel, MRI data (2D maximum intensity projection). Lower panel, 3D surface render. Scale bar: 20 mm. **(c)** Maize roots imaged using MRI-PET [24]. Two plants are growing in the same pot. The greyscale image is MRI, the colour is ^{11}C PET data following application to a leaf of one plant. **(d)** OpenSimRoot [65] simulation using output from **(a)** to model rhizosphere N depletion. **(e)** Maize root imaged at 9 DAS using optical projection tomography (OPT) and PET [4]. The black and white image is OPT, the colour is ^{11}C PET data.

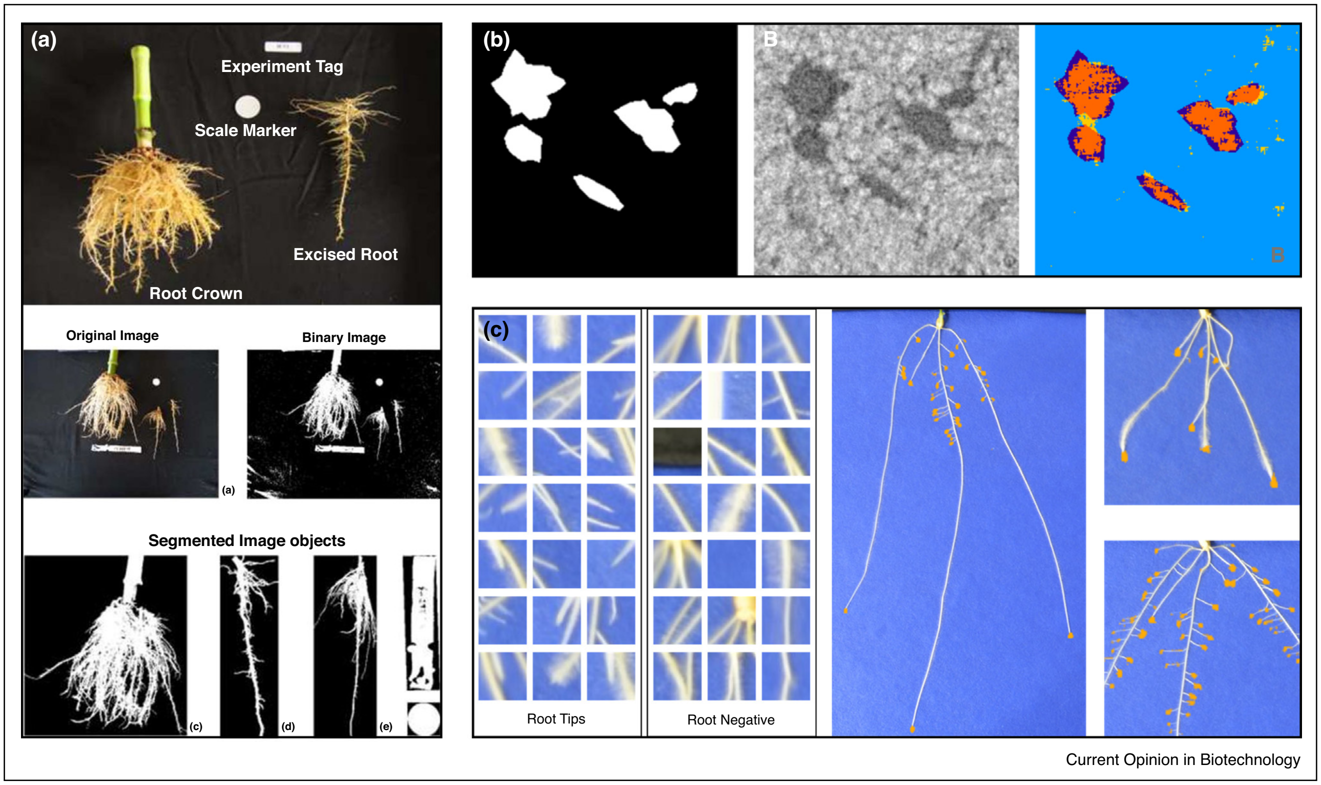
phenotyping tool, perhaps in combination with other geophysical sensors.

Advances in root image analysis

As high-throughput image capture of root systems has become mainstream and generates ever larger datasets, there is a requirement for fast and accurate software solutions to reliably derive traits. Until fairly recently, software has focused on 2D imaging paradigms, resulting in a large number of tools, exhibiting a mixture of manual (e.g. DART [49]), semi-automatic (e.g. SmartRoot [50], RootNav [51]), and fully automated (e.g. EZ-Rhizo [52], GiA Roots [53], DIRT [40]) approaches (Figure 3). Software in this area has traditionally relied heavily on the assumption that root images are consistent across an experiment, and that the root system exhibits high contrast against the background. Where this is the case, image thresholding to identify root material as reliably lighter or darker than the background has proven effective [51,53]. Thresholding alone can be susceptible to image noise, where image pixels are incorrectly assigned as either root material, or background. It has been common practice to perform corrective filtering of segmented images prior to analysis of the RSA. Morphological operations such as erosion and dilation can be used to correct small errors, and skeletonisation may be used to simplify the root structure to make topological analysis and trait measurement more straightforward. [52,53] are examples of tools that take this approach.

Following the identification of root material within an image, RSA traits such as width are easily derived. Some tools, such as EZRhizo [52] and WinRhizo [54] utilise pixel-distance transforms to approximate the width of each root, deriving a frequency distribution of root size within an image. These tools operate automatically but become less

Figure 3



Automated root image analysis software. **(a)** DIRT [40] measures traits based on the 'shovelomics' approach [37]. Root systems are washed, and imaged from above in front of a dark background. Root systems are separated from background via thresholding, and RSA traits derived from each segmented object. **(b)** Root-soil segmentation in X-Ray CT [61]. Root and soil pixels are identified via a Support Vector Machine classifier trained on deep-learned features. Images show the ground-truth, original image, and SVM classifier output. **(c)** End-to-end deep learning for root tip identification [59•]. A deep network trained on thousands of instances of root tips and negative samples can be passed over an entire image to obtain likely root tip locations.

reliable where root systems exhibit complex topology, including bunched roots and crossovers. Some tools have attempted to "track" the root system, maintaining a link between the bases and apices of roots to form coherent geometries rather than isolated pixels. RootTrace [55], for example, uses a particle filtering framework to track *Arabidopsis* roots from base to apex on agar plates. SmartRoot [50] uses a semi-automatic approach to follow root material once first identified by a user. RootNav [51] is also semi-automated, seeking the shortest path on the image between user-identified seed and root tip locations.

3D root analysis software is less developed, with fewer tools available. Multi-view reconstruction from RGB images has been applied to roots grown in transparent media [2,3•]. For MRI data, [56] used Frangi filters to highlight tubular structures in the 3D image, before applying a shortest path search to obtain root topology. The root structure is then cleaned using a tree-pruning approach. In X-ray CT images, RooTrak [57] utilises a level set tracking approach to follow roots within a soil column and has been extended to handle multiple competing root systems [58].

Deep machine learning is becoming a standard technique for many computer vision problems as large annotated datasets become available. Within plant science, the majority of deep learning has been applied to plant shoots. For 2D root images, tip locations have been identified using a deep network-based classifier, scanned over an image to produce a location map [59•]. After training, deep learning algorithms delivered impressive improvements in tip detection (>99%) compared other approaches (<60%) [59•]. Deep learning thus has the potential to reduce the reliance on user-input to increase both the throughput and quality of phenotypic data [60]. For 3D images, deep learning has been applied to the root-soil segmentation problem, where deep learned features are used to drive a Support Vector Machine classifying root/soil pixels [61].

Future perspectives

This review has focused on recent advances in phenotyping plant root architecture, particularly in the field of non-destructive 3D imaging of root systems. The next challenge is to incorporate these techniques into phenotyping platforms with throughputs comparable to 2D systems to allow large-scale quantitative genetic studies. Selecting crops based on their root anatomical traits also represents a promising approach. For example, maize lines with increased root airspaces (root cortical aerenchyma; RCA) grown under drought stress in smallholder plots in Malawi had 78–143% greater yield than crops with less RCA [62•]. Similarly, root xylem diameter has been linked with conservation of water resources to aid grain filling in wheat [63•]. To date, screening for root anatomical traits is very time consuming. However, the recent development of high-throughput 2D and 3D anatomical phenotyping approaches employing microtoming [64] and laser ablation tomography [62•], respectively, makes it possible to profile the thousands of root samples required for breeding programmes.

Advances in data acquisition, handling and processing are becoming increasingly important to plant phenotyping [65]. Integrating innovative approaches such as deep learning into root phenotyping pipelines will require researchers to actively engage with computer scientists. Similar challenges and opportunities face researchers wanting to employ *in silico* models to probe how multiple root architectural and anatomical traits interact to impact plant performance. The development of open-source software such as OpenSimRoot and RootBox [66•,67,68] promises to greatly aid modelling efforts by root researchers and provide invaluable insight into the highly non-linear interactions between root phenotypes. Such multi-disciplinary approaches will underpin efforts to develop crops with improved root systems and help address the urgent need for future crops better adapted to the challenge of climate change.

Conflicts of interest statement

None declared.

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