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Current Opinion in Biotechnology

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

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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.

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Current Opinion in Biotechnology 2019, **55**:1–8

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Saulius Klimasauskas** and **Linas Mazutis**

https://doi.org/10.1016/j.copbio.2018.06.002

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.

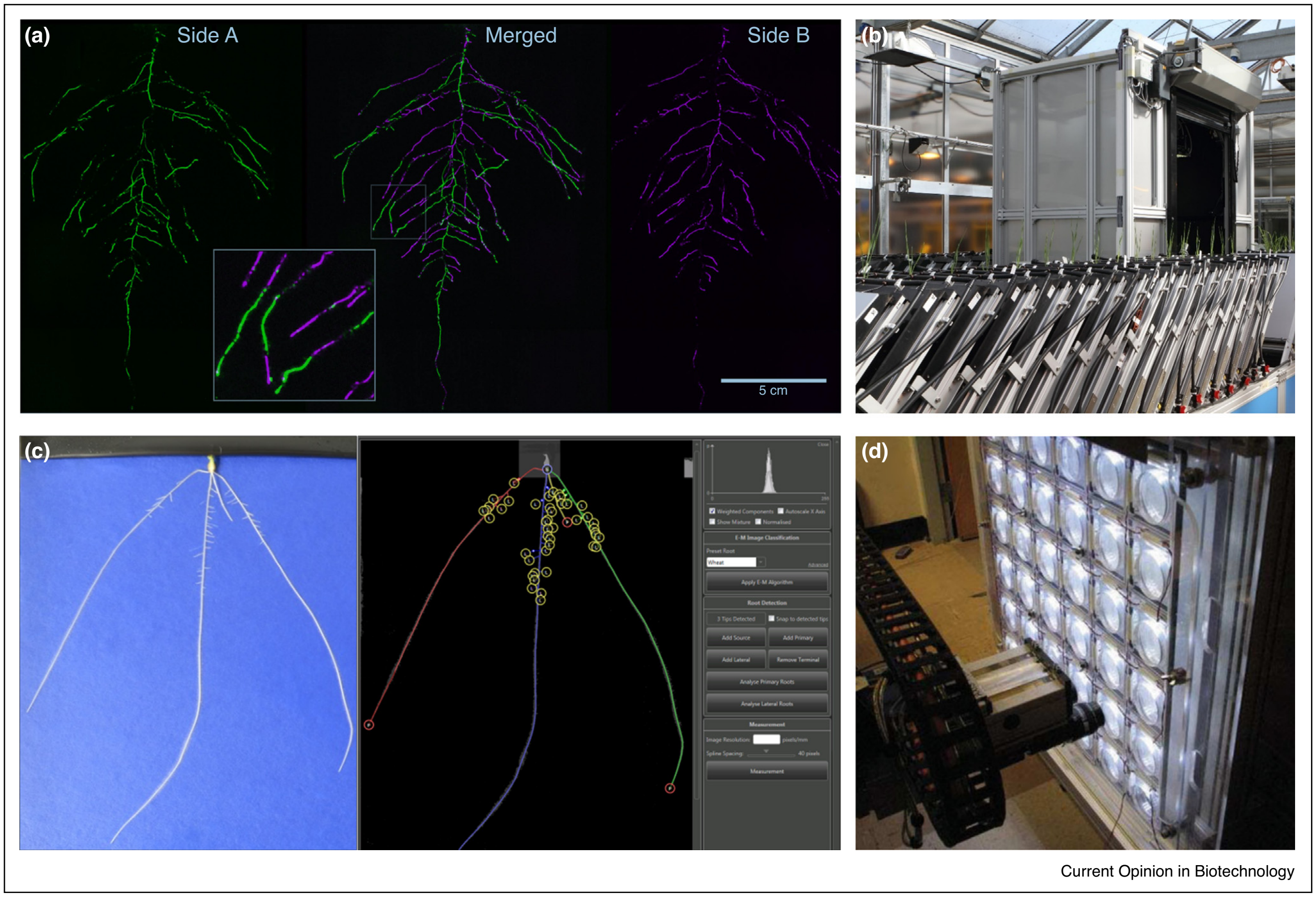
This review discusses recent advances in root phenotyping. To date, classical non-destructive 2D techniques such as agar plates or rhizotrons have been integral to our understanding of root development (Figure 1). Non-destructive analysis of 3D root growth is also possible using transparent gels [2,3•,4] 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



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,