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Abstract

Root system architecture determines crop capacity to acquire water and nutrients in the dynamic and variable soil environment. Increasing attention is paid to searching for optimal root traits to improve resource uptake efficiency and adaptation to heterogeneous soil conditions. This chapter summarises genetic variability and plasticity in root traits relevant to increased efficiency of soil resource acquisition. Approaches available for high-throughput phenotyping of root architecture traits at both laboratory and field scales are critically assessed. The advent of several novel imaging technologies such as X-ray computed tomography coupled with image-analysing software packages offers a great opportunity to non-invasively assess root architecture and its interactions with soil environments. The use of three-dimensional structure–function simulation root models is complementary to phenotyping methods, providing assistance in the crop breeding programmes. We also discuss applications and limitations of these novel visualisation technologies in characterising root growth and the root–soil interactions.

Keywords

Root traits • Variability • Plasticity • Hydroponics

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8.1 Introduction

Root system is a fundamental component of plants. Apart from mechanical support to the above-ground parts, root system is also the major organ for foraging and acquiring nutrients and water from the surrounding soil. Root system is a complex three-dimensional (3D) structure exhibiting a specific spatial and temporal configuration of root types. Root system architecture (RSA) refers to in situ spatial distribution of the root system within the rooting volume (Hinsinger et al. 2011; Lynch 1995, 2007; Manschadi and Manske 2013). RSA plays a vital role in the exploration of soil zones and acquisition of soil water and nutrients (Gregory et al. 2009; Hammond et al. 2009; Lynch and Brown 2012). It is plastic and dynamic, allowing plants to respond to their environments in order to optimise acquisition of important soil resources (Zhu et al. 2011).

Studies of RSA are concerned typically with an entire root system of an individual plant, rather than just fine details of the root structure (Lynch 1995). The root architectural traits include three general categories: topological properties (describing the pattern of root branching), geometric properties (the presence of roots in a spatial framework, such as the growth angle of root axes) and physiological properties (such as root growth rate, root exudation and root water and nutrient use efficiency) (Chen et al. 2011a; Gregory 2008; Manschadi et al. 2008). Crop adaptation to suboptimal soil conditions is dependent on RSA, and thus crop survival and fitness are determined by the RSA (Eshel and Beeckman 2013; Fitter et al. 2002; Lynch 1995). However, as ‘the hidden half’ of a plant, root system is often underappreciated largely due to the inherent difficulty of accessing it for studies (Eshel and Beeckman 2013; Smith and De Smet 2012).

Exploiting genetic diversity in root traits associated with acquisition of scarce soil resources and adaptation to edaphic stresses can significantly enhance resource use efficiency in crop plants and thus lead to improved productivity. It might be advantageous for a plant to have the root system

with architectural traits specifically adapted to the prevailing soil conditions (Trachsel et al. 2013). The identification of relevant root traits offers the potential to increase the grain yield of not only crops growing soil resources but also crops growing with optimal water and nutrient supply by revealing physiological traits associated with the partitioning of dry matter.

The identification of optimal root traits under stress environments depends on targeting the probable stresses that the crop may face during the growing season. Targeted development of crop genotypes with increased efficiency of nutrient capture (Rengel 2005; Wu et al. 2005) and water use (Kamoshita et al. 2000; Liu et al. 2007; Manschadi et al. 2006; Ober et al. 2005; Rengel 2013) relies on a better understanding of root structure and functions and the exploration of optimal root traits for specific growth environments (Wang and Smith 2004). It has been demonstrated that modification of root architectural traits could contribute to improved grain yield, drought tolerance and resistance to nutrient deficiencies (Beebe et al. 2006; Steele et al. 2006; Tuberosa et al. 2002a).

Quantitative genetic studies require efficient phenotyping protocols (Trachsel et al. 2013). However, the inability to efficiently and accurately phenotype large mapping populations has been a key impediment to wide-scale use of root-related genetic information in breeding (Chen et al. 2011a; De Dorlodot et al. 2007). Hence, accurate phenotyping of root-related traits is one of the most important practices for translating into breeding programmes the recent physiological and genetic advances in understanding the role of root systems in improving crop yield and productivity in dry environments. However, phenotyping of root traits requires multidisciplinary analysis because the root structure and function and their responses to heterogeneous soil environments are dynamic and complex (Doussan et al. 2003; Hodge 2004; Pierret et al. 2006; Valizadeh et al. 2003). Hence, phenotyping for optimal root traits is often conducted under controlled environmental conditions, whereas systematic phenotyping for root traits in the field remains challenging (Fiorani and Schurr 2013; Trachsel et al. 2011).

In the recent decade, some novel non-invasive and high-throughput phenotyping technologies have been developed for fast, accurate and robust analyses of root structure and function. In addition, current simulation computer models offer a promise in characterising intrinsic genetic properties and phenotypic plasticity of root traits in large-scale phenotyping required in breeding for improved productivity (Dunbabin et al. 2013; Struik and Yin 2007).

This chapter discusses genetic variability and plasticity in root traits relevant to increased soil resource use efficiency and better adaptation to specific soil environments, followed by an overview of recent developments in high-throughput phenotyping methods. This review also highlights applications and limitations of some novel visualisation technologies and modelling simulations in characterising root growth and the root–soil interactions.

8.2 Root Trait Variability

Genotypic variability and phenotypic plasticity are the two general types of variability in root architectural traits. These are derived from two different developmental pathways, namely, genetically determined intrinsic pathway (governing the basic architecture and the limits of plasticity) and environmentally triggered responsive pathway (Malamy 2005). These two pathways combine in intricate ways to create a highly complex 3D root structure influenced by genetics as well as the availability of resources in the heterogeneous soil environment (Baddeley et al. 2007).

8.2.1 Genotypic Variability in Root Traits

Variation in root architectural traits critically influences the capacity and efficiency of a plant in foraging and taking up water and nutrients from soil. For example, changes in the root system architecture in response to low phosphorus (P) availability may enhance P uptake (Nielsen et al. 2001). Shallow rooting is beneficial for P

uptake because in many soils, most of the available P is retained in the topsoil layers. In soybean (*Glycine max*) genotypes, increased yield potential was linked to increased capacity to take up water from deep (1.1 m) soil horizons in the field (Ober et al. 2005); similar connection was established for upland rice (*Oryza sativa*) in Laguna, Philippines (Kamoshita et al. 2000; Kondo et al. 1999), and wheat (*Triticum aestivum*) in Western and Southern Australia (Wong and Asseng 2006; Manschadi et al. 2010). Understanding the role of and manipulating root length branching at depth and seminal root angles have been flagged as key factors likely to underpin further increases in wheat yield (Manschadi et al. 2010).

Our recent studies examined genotypic variability in a large germplasm collection of narrow-leafed lupin. Wild genotypes with contrasting root architecture differed in root growth, root distribution in the profile and P acquisition in response to localised P supply (Chen et al. 2013a). Selected genotypes differed in root length density (root length in a unit soil volume).

Specifically selecting for improved root traits, such as root proliferation at depth, may contribute to increased productivity in crops, especially in dry soil conditions, and in soils with high strength because of natural settling or formation of a shallow hardpan due to vehicle movement (Hall et al. 2010). This is particularly important because attempts to increase root density at depth using agronomic approaches (e.g. deep fertiliser placement and ripping) have been largely unsuccessful (e.g. Baddeley et al. 2007).

There is little knowledge on genotypic variability in root function related to the architecture. For example, wild genotypes of narrow-leafed lupin (*Lupinus angustifolius*) exhibited genetic variation in exudation of organic acid anions into the rhizosphere (Chen et al. 2013b). We observed that, at optimal P, the large-rooted genotype exuded citrate, acetate and malate, whereas the other two genotypes with smaller root systems only released citrate in significant amounts. The significance of these findings in a relationship between root architecture and functions is yet to be assessed.

8.2.2 Phenotypic Plasticity in Root Traits

Several mechanisms may be involved in regulating root architecture alteration in response to heterogeneous nutrient availability. Examples of such mechanisms are root proliferation in localised nutrient-rich patches, changes in rooting depth or the relative length of different root diameter classes (e.g. Dunbabin et al. 2001a; Ho et al. 2005; Paterson et al. 2006; Smith and De Smit 2012). Plant changes in root morphology in response to changes in P availability are an essential strategy for efficient P acquisition (Chen et al. 2013b; Lambers et al. 2011).

Crop genotypes often exhibit variable capacity to alter the growth, number, size and distribution of the root systems in order to optimise resource capture when exposed to heterogeneous environmental conditions. Phenotypic plasticity was evidenced in root length density in the lupin genotypes in response to varying P supplies (Fig. 8.1; see also Chen et al. 2013a). For example, in comparison with the nil-P treatment, the large-rooted genotype produced significantly more branches in the top 30 cm of the soil profile when P was placed in a band 10 cm below the soil surface. Such developmental plasticity is based on the capacity of plant cells either to remain undifferentiated until the root-growth response is initiated or to dedifferentiate into cells that can grow into new roots.

In terms of root mass allocation, many *Lupinus* species with a high capacity to acquire P exhibit low root-growth plasticity at low P supply (Pearse et al. 2006). On the other hand, some *Lupinus* species increase root–shoot biomass ratio during water stress (Carvalho et al. 2004).

8.3 Phenotyping Systems

Crop breeders and researchers are showing increased interest in phenotyping for root architecture traits as part of their breeding programmes. High-throughput phenotyping for

root architecture traits requires fast, reliable and accurate root observations and measurements. Recent progress in the development of root-related methodologies, from traditional excavation to modern non-destructive imaging technologies including X-ray computed tomography (CT), has significantly enhanced our capacity to visualise, quantify and conceptualise root architecture and its relationship to (1) crop adaptation to variable growth environments and (2) plant productivity (Gregory et al. 2009; Iyer-Pascuzzi et al. 2010; Lynch 1995). It is still a challenge to elucidate the genetic and developmental basis of the root system architecture, and a combination of laboratory- and field-based approaches should be considered (Clark et al. 2011). In this section, we overview current phenotyping systems employed in the controlled environments and the field (Table 8.1) and discuss their applications and limitations.

8.3.1 Controlled Environments

A number of phenotyping approaches are available for (1) destructive and (2) non-destructive sampling of root systems. Destructive sampling by excavating whole root systems from soil-filled containers (e.g. pot, columns, boxes, tubes and chambers) is used commonly in root studies in the controlled environments (Table 8.1). In the recent decades, non-destructive approaches have been developed for high-throughput phenotyping of root architecture traits with the support of advanced optical recording techniques. These include (1) soil-filled rhizotrons with clear panels for root observations and (2) soil-free approaches in artificial media, such as hydroponics, aeroponics and the gel chamber or agar-plate systems.

8.3.1.1 Soil-Filled Pots

The soil-filled pots provide environments for crop growth that may to some extent simulate those in the field. Various root traits can be measured, including total root length, root mass and root density per soil volume; also, roots at various depths in the soil profile can be measured

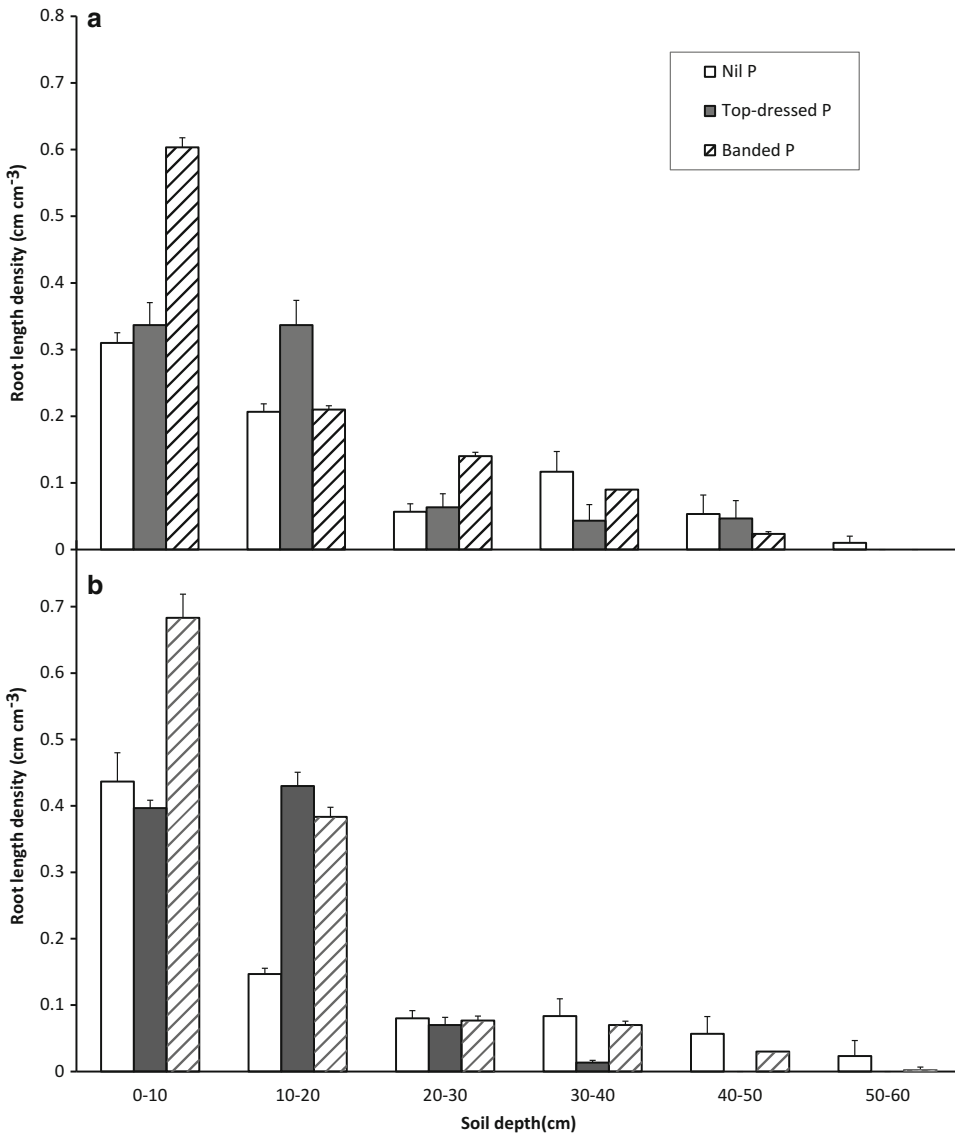


Fig. 8.1 Variability in root length density (cm cm^{-3}) in the soil profiles of two wild genotypes (**a** #071; **b** #085) of *Lupinus angustifolius* grown in a glasshouse for 42 days under three P-application treatments: nil P (no fertiliser P

application), top-dressed P (fertiliser P applied on the soil surface) and banded P (fertiliser P placed in a narrow band 10 cm below the soil surface). Data are means + s.e. ($n=73$) (Modified from Chen et al. 2013a)

(Araki and Iijima 1998). However, this method requires destructive sampling of roots and involves the process of root washing out of soil (Hund et al. 2009b). Hargreaves et al. (2009) placed a starch-based polymer net sac in the centre of the soil-filled plastic pot and observed genotypic variation in root numbers, length, mass and root angles (i.e. vertical spread) of lateral

roots in five barley genotypes. The soil sac method could be improved to allow more systematic construction and data collection. However, this method, similar to the standard pot method, is destructive. Despite all due care, roots could be broken inside the netting so their coordinates could not be taken, jeopardising measurements of root spread.

Table 8.1 Summary of methods for phenotyping root traits

Growth medium	Phenotyping system/method	Crop species	Capacity	Traits observed	Merits	Limitations	Example reports
Agarose gel or gellan gum	Agar-solidified nutrient solution in Petri dishes; transparent gel or gellan gum on transparent plastic plates or in ungraduated borosilicate cylinders or glass cylinders/chambers	Barley (<i>Hordeum vulgare</i> , <i>H. spontaneum</i>), rice (<i>Oryza sativa</i>), canola (<i>Brassica napus</i>), <i>Arabidopsis</i> (<i>Arabidopsis thaliana</i>), timothy (<i>Phleum pratense</i>), tomato (<i>Lycopersicon lycopersicum</i>), lettuce (<i>Lactuca sativa</i>), alyssum (<i>Aurinia saxatilis</i>)	Up to a large number of genotypes (hundreds)	Root length, number, elongation rate; vertical angular spread of roots; root hairs, diameter, surface area, volume, distribution; relative elongation rates between the elongation zone and the meristem; some 27 static/dynamic and global/local root traits calculated with the RootReader3D software based on 3D images	The gel/gellan-gum system provides easy, fast, non-destructive and repeated measurements of 2D (via a flatbed scanner) or 3D (using RootReader3D software) root system. It can be applied to hundreds of plants, to create a uniform and reproducible environment for root growth, easy handling with minimal risk of accidental damage to roots. The 2D analysis is simple to record and analyse and would be simple to model. The use of gellan-gum systems has an enormous potential for high-throughput root phenotyping and novel trait discovery	Anaerobic conditions in agar may alter plant growth. The 2D system with artificial environments may not reliably represent the 3D soil environment. The size of the chamber may restrict its use to studies of young seedlings only. Efforts to expand the 2D images into 3D structure remain constrained by low throughput (>1 h to image a single root system), small scanning volumes and limited quantification capabilities	Bengough et al. (2004), Clark et al. (2011), Fang et al. (2009), Hargreaves et al. (2009), Iyer-Pascuzzi et al. (2010), Shiet al. (2013), van der Weele et al. (2003)
Nutrient solution	Hydroponics (roots in solution); semi-hydroponics (roots in air and moisture provided by automatic irrigation system)	Maize (<i>Zea mays</i>) narrow-leaved lupin (<i>Lupinus angustifolius</i>)	Up to a large number of genotypes (hundreds)	Root length, number, elongation rate, hairs, diameter, surface area, volume, mass	High throughput; can reproduce 2D or 3D root systems; simple and fast measurements of dynamic root growth. The semi-hydroponic platform permits non-invasive, repeated imaging of	Plants may perform differently in hydroponics and soil. Hydroponic method does not reflect heterogeneous distribution of nutrients often present in real soils	Chen et al. (2011a, 2012), Sanguinetti et al. (2006)

dynamic root growth and allows phenotyping of relatively large sets of genotypes for a long growth period in a small space	Basu et al. (2007), Hund et al. (2009a, b), Liao et al. (2004)	Root growth is forcibly two dimensional. Phenotyping large sets of genotypes beyond early growth stages remains problematic
Simple, non-destructive, 2D observation of root growth during the early days. Root growth receives lower mechanical impediment than in soil. The technique can be used to analyse kinematics of various root systems using KineRoot image-analysing software	The soil sacs provide a simple method to compare root growth against the gel chambers and X-ray pots. The X-ray method allowed in situ non-destructive and sequential data collection. An advantage of the X-ray scanner was its facility to track root growth inside the soil throughout the pot, showing clearly that the angles of roots change during growth	The excavation method remains labour intensive and does not allow for high throughput. Artificial systems fail to mimic the complex interaction between the plant and intrinsic abiotic and biotic soil properties. The soil sac method is destructive and data are only obtained where the roots pass through the net boundaries. Roots may be broken inside the netting. It is also
dynamic root growth and allows phenotyping of relatively large sets of genotypes for a long growth period in a small space	Basu et al. (2007), Hund et al. (2009a, b), Liao et al. (2004)	Root growth is forcibly two dimensional. Phenotyping large sets of genotypes beyond early growth stages remains problematic
The paper pouches are placed upright into plastic containers with nutrient solution	Common bean (<i>Phaseolus vulgaris</i>); maize (<i>Zea mays</i>)	Root length of basal roots; taproot length per vertical layer; growth angles of basal roots (reflecting root gravitropism); lateral root length and number; other common root traits obtained via WinRhizo software based on 2D root images
Small number of genotypes	Common bean (<i>Phaseolus vulgaris</i>); maize (<i>Zea mays</i>)	Root length of basal roots; taproot length per vertical layer; growth angles of basal roots (reflecting root gravitropism); lateral root length and number; other common root traits obtained via WinRhizo software based on 2D root images
PVC or Perspex cylinders/columns, tubes or boxes or transparent rhizoboxes filled with soil or artificial solid substrate; soil sacs consisting of a plastic pot with a starch-based polymer net sac; root growth assessed via 2D imaging by a camera or flatbed scanner, 3D image by X-ray scanner, or excavation	Barley (<i>Hordeum vulgare</i>), maize (<i>Zea mays</i>), rice (<i>Oryza sativa</i>), wheat (<i>Triticum aestivum</i>)	Root number, length (at depth), vertical spread angles, cortical aerenchyma
Small number of genotypes	Barley (<i>Hordeum vulgare</i>), maize (<i>Zea mays</i>), rice (<i>Oryza sativa</i>), wheat (<i>Triticum aestivum</i>)	Root number, length (at depth), vertical spread angles, cortical aerenchyma
Sand, soil or artificial solid substrates (laboratory)	Araki and Iijima (1998), Hargreaves et al. (2009), Hund et al. (2009a), Watt et al. (2006), Zhu et al. (2010)	The excavation method remains labour intensive and does not allow for high throughput. Artificial systems fail to mimic the complex interaction between the plant and intrinsic abiotic and biotic soil properties. The soil sac method is destructive and data are only obtained where the roots pass through the net boundaries. Roots may be broken inside the netting. It is also

(continued)

Table 8.1 (continued)

Growth medium	Phenotyping system/method	Crop species	Capacity	Traits observed	Merits	Limitations	Example reports
						possible that the netting could impede growth as roots may face resistance on reaching the netting, forcing deviation or diameter expansion. The X-ray pots required some patience to set up accurately	
Soil (field)	Excavation/shovelomics; installation of minirhizotron in soil	Maize (<i>Zea mays</i>), common bean (<i>Phaseolus vulgaris</i>), narrow-leaved lupin (<i>Lupinus angustifolius</i>)	Small to medium number (up to 50) of genotypes	Root cortical aerenchyma, length, number at depth; branching density of brace roots; root angles;	Provide real plant growth environments; shovelomics techniques provide high-throughput assessment of the tested crop species	Application of shovelomics techniques for other crop species needs further examinations. These methods remain labour intensive. Accurate analysis is also compromised due to the loss of some fine and broken roots during the process. Information on root architectural properties	Araki et al. (2000), Chen et al. (2014), Postma and Lynch (2011), Trachsel et al. (2011), Zhu et al. (2010)
Soil (field)	DNA-based quantification method (qRT-PCR typing)	Wheat (<i>Triticum aestivum</i>); rice (<i>Oryza sativa</i>)	Small to medium number (up to 50) of genotypes	Root DNA density in soil	High throughput	DNA-based techniques only detect and quantify root mass presented in soil samples	Huang et al. (2013), Topp et al. (2013)

8.3.1.2 Rhizotrons

Root observation rhizotrons (rhizoboxes or root chambers) are similar to the soil-filled pot method mentioned above, except that clear acrylic glass panels allow visual monitoring of root growth at the surface of the glass (Manschadi et al. 2006, 2008; Wiese et al. 2005). Variation in root growth and morphology among the tested crop plants can be traced on the outside surface of the acrylic glass using a marker pen – different colours may be used to indicate the presence of roots at successive time intervals, followed by photographing or scanning for root quantification (Fig. 8.2).

Rhizotrons can be designed and constructed to meet specific research needs, such as for deep-rooted crops and long growth periods. For example, Manschadi et al. (2006) used 240-cm-wide, 120-cm-deep root chambers in examining spatial patterns of root length distribution in a drought-tolerant wheat genotype compared with the standard wheat variety at anthesis. Rhizotron systems artificially restrict root growth to two dimensions only. In addition, they suffer from the general disadvantages of pot experiments associated with the disturbed soil structure, altered root-zone temperatures and the limited rooting volume.

8.3.1.3 Agar (Gel, Gellan-Gum) Systems

The agar/gel method, a useful non-soil system, allows quick and easy measurement and visualisation of dynamics of early root growth in seedlings. Root architecture traits can be non-destructively recorded in two dimensions (2D) or 3D using flatbed scanning, digital cameras or X-ray cameras through the transparent substrate. This method has been used for high-throughput phenotyping of root architecture traits in various crop species, such as barley (wild, *Hordeum spontaneum*; domesticated, *H. vulgare*) (Bengough et al. 2004; Hargreaves et al. 2009) and rice (*Oryza sativa*) (Clark et al. 2011; Iyer-Pascuzzi et al. 2010).

The agar/gel method permits detailed characterisation of root traits and root development; for example, the pattern and timing of lateral root initiation were characterised in *Arabidopsis* seedlings (Dubrovsky et al. 2006).

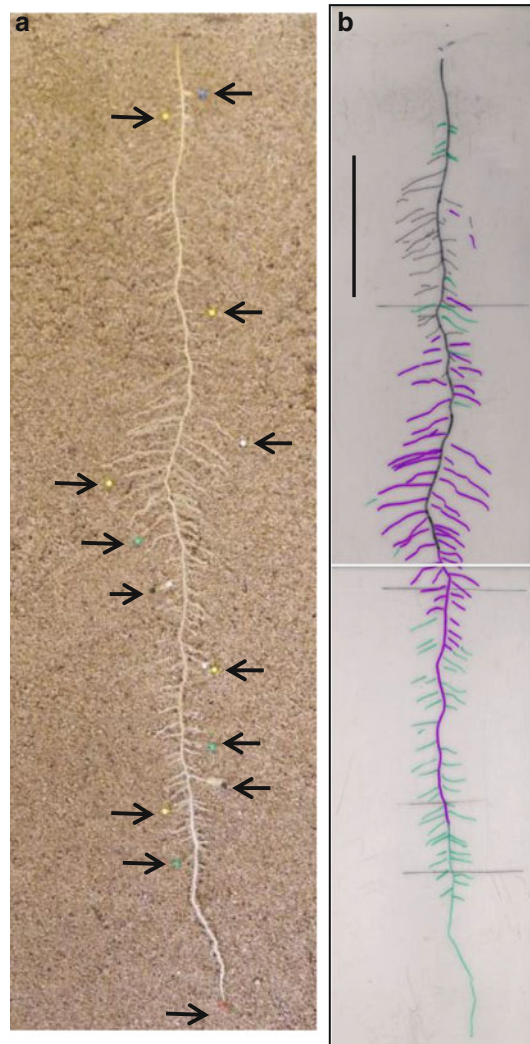


Fig. 8.2 Example of two-dimensional root images of a wild genotype of narrow-leaved lupin (*Lupinus angustifolius*) grown in a soil-filled rhizotron as part of a phenotyping experiment to determine genotypic variability in root growth and temporal–spatial exudation among wild and commercial varieties. (a) A rhizotron with the acrylic glass panel removed to expose root system for sampling root exudates around individual root tips using the anion exchange membrane (AEM) indicated by inserted pins and arrows. (b) Root image of the same plant is (a) acquired by a flatbed scanner via scanning the acrylic glass with traced root morphology at three consecutive times: 14 (black), 20 (purple) and 26 (green) days after sowing (Bar = 10 cm) (Chen and Rengel 2014)

A new algorithm for computational image analysis of deformable motion at high spatial and temporal resolution was applied to contrasting root growth in *Arabidopsis*, timothy (*Phleum pratense*), tomato (*Lycopersicon lycopersicum*), lettuce (*Lactuca sativa*) and alyssum (*Aurinia saxatilis*) (van der Weele et al. 2003) using the gel system.

By incorporating image-analysing tools, e.g. RootTrace (see Sect. 1.4; Table 8.2), the agar/gel method enables high-throughput, automatic tracing of root growth in *Arabidopsis* seedlings to quantify root length, curvature and stimulus response parameters such as onset of gravitropism (French et al. 2009). The gel plate system offers a moderately rapid screening method for seedlings, the results of which appear to reflect angular root spread in 3D, though care must be taken to avoid contamination problems and use of inappropriate media. The gel plate method is also of particular benefit for non-destructive monitoring of seedling root growth. Hargreaves et al. (2009) compared root growth in gel chambers, soil sacs and X-ray microtomography pots and arrived at similar (albeit not identical) ranking order of angles using the three methods.

Earlier, limitations of the gel chamber system have been outlined (see Futsaether and Oxaal 2002; Hargreaves et al. 2009). These include the chamber size, restricting the method to studies of young seedlings and the fact that it is an artificial environment. Moreover, gel chambers generate 2D data as opposed to the 3D environment encountered by most plants, and anaerobic conditions in agar may alter plant growth (Clark et al. 1999; Hargreaves et al. 2009).

The use of gellan-gum growth systems with superior optical clarity also facilitates non-invasive 2D (Iyer-Pascuzzi et al. 2010) and 3D (Fang et al. 2009) imaging and temporal studies of root systems while allowing reproducible control of the rhizosphere. Topp et al. (2013) employed nutrient-enriched gellan gum to grow rice and demonstrated the capacity of a semiautomated 3D *in vivo* imaging and digital phenotyping pipeline to interrogate the quantitative genetic basis of the root system. The study

phenotyped 25 root traits governing the distribution, shape, extent of exploration and the intrinsic size of root networks at three observation times during the seedling stage. While these recent studies demonstrate the use of gellan-gum systems for potential high-throughput root phenotyping and novel trait discovery in 2D, efforts to expand these investigations into the 3D structure remain constrained by the long scanning times, small scanning volume and limited quantification capability (Clark et al. 2011). Hence, the agar/gel and gellan-gum methods work well for simple root systems, but obscure the more complex 3D root architectures.

8.3.1.4 Hydroponics

Growing plants hydroponically is widely used in seedling studies. The inexpensive, space-saving, high-throughput (semi-) hydroponic system offers the advantage of growing a large number of plants under uniform conditions, two important prerequisites for investigating quantitative traits, particularly those of low heritability. Measuring traits at the seedling stage in hydroponic culture eliminates the challenges of soil contamination and root loss during washing (Chen et al. 2011a).

Seedling traits of 47 commercial maize (*Zea mays*) hybrids were screened using nutrient solution (Sanguineti et al. 2006). Singh et al. (2013) reported a new hydroponic phenotyping technique in examining survivability and drought tolerance of 15-day-old seedlings of 80 genotypes of lentil (*Lens culinaris*).

A novel semi-hydroponic phenotyping platform developed by Chen et al. (2011a) has the potential in studying root response and plasticity in morphological and physiological responses to water and nutrients because the supply of water and nutrients can be adjusted easily. This high-throughput phenotyping system was designed for characterising variability in root architectural traits of narrow-leaved lupin (Chen et al. 2011a, b, 2012). The system is based on a 240-L mobile bin and allows root growth up to 1-m depth, with repeated observations and measurements of 2D root structure without the need for destructive sampling (Fig. 8.3). It is notable that this system

Table 8.2 Selected software packages for root image analyses

Software	Brief descriptions	Root traits	Licence	Automation level	Plant requirement	Image requirements	Source	Website
DART	DART (Data Analysis of Root Tracings) can display root growth sequences over time. It generates structured and flexible datasets of individual root growth parameters and thus enables studying root system architecture	Root length; topological properties; insertion	F	Manual	Seedlings to mature plants	Any	Le Bot et al. (2010)	www.avignon.inra.fr/infos/404
Delta-T-Scan	The software package allows fast and accurate root measurements, including root tip count, with high resolution over large area and no calibration required	Root length, surface area and volume; root tip count and location	C	Semiautomated	Any	misp, bmp, tif, pex	Delta-T website	www.delta-t.co.uk/groups.html?group2005092301354
EZ-Rhizo	EZ-Rhizo enables the analysis of images of root growth in 2D (e.g. on agar plates). It permits root detection and measurements of multiple root architecture traits. It is a good tool for developmental and phenotypic descriptions of individual plants and genotypes and for root growth under varying nutritional and environmental conditions	Root length; insertion and insertion angle; number of branches; topological properties	F	Semiautomated	Arabidopsis	bmp	Armengaud et al. (2009), Armengaud (2009)	www.ez-rhizo-psrg.org.uk/

(continued)

Table 8.2 (continued)

Software	Brief descriptions	Root traits	Licence level	Automation level	Plant requirement	Image requirements	Source	Website
GiA roots	<p>GiA Roots (General Image Analysis of Roots) is capable of automating the large-scale analysis of root networks. It helps users easily quantify root structure</p>	<p>Root length, surface area and volume; number of branches; root depth; convex hull</p>	F	Automated	Seedlings to mature plants	Any	Galkovskiy et al. (2012)	www.rootnet.biology.gatech.edu/giaroots/download/signup.php
GROWSCREEN-Root	<p>GROWSCREEN-Root provides automatic analysis of root architecture. Images of whole root systems grown in agar-filled dishes are acquired with a CCD-camera. The key element of the software is the extraction of a tree model for a root system</p>	<p>Root length; insertion angle; number of branches</p>	OD	Semiautomated	Any	Any	Nagel et al. (2009)	www.fz-juelich.de/ibg/ibg-2/EN/methods_jppc/GROWSCREEN_root/_node.html
Growth Explorer	<p>Growth Explorer is designed for analysing root growth patterns from 2D time-series images, treating them as a spatio-temporal 3D image volume</p>	<p>Velocity profile</p>	OD	Automated	Any	Any	Basu and Pal (2012)	www.plant-image-analysis.org/software/growthexplorer
IJRhizo	<p>IJRhizo is an ImageJ macro for the batch processing of scanned images of root samples physically separated from soil by washing. It automatically generates root length and radius measurements</p>	<p>Root length and diameter; length of primary roots; total number and length of lateral roots; angle of branching</p>	O&F	Automated	Any	jpg	Pierret et al. (2013)	www.plant-image-analysis.org/software/IJ_Rhizo

ImageJ	ImageJ is a public domain Java image-processing program. It can measure distances and angles and calculate area and pixel value statistics of user-defined selections. It supports standard image processing functions. Spatial calibration is available to provide real-world dimensional measurements in units	Root length; root area; distance between root nodes; branching angles	O&F	Semiautomated	Any	Any	Kimura et al. (1999), Tajima and Kato (2011)	rsb.info.nih.gov/ij/
Root System Analyser	Root System Analyser package analyses 2D images or image sequences of roots. It requires MATLAB to run	Root length and diameter; insertion and insertion angles; root tip count; root connectivity; inter-root distances; branching angles	F	Automated	Any	Any, segmented	Leitner et al. (2014)	www.csc.univie.ac.at/rootbox/rsa.html
RootDetection	RootDetection detects single-strand roots traces their paths and measures the resulting length automatically. It also provides tools for manually tracing features such as hypocotyls or side roots	Root length; tracing roots	F	Automated; semiautomated; manual	Any	Any		www.labutilis.de/rd.html
Rootfly	Rootfly is specifically designed for analysing minirhizotron images. It can track root development and determine root lifespan	Root length and diameter; colour; root initiation and death rates	O&F	Manual	Seedlings to mature plants, minirhizotron	jpg, bmp	Zeng et al. (2008)	www.ces.clemson.edu/~stb/rootfly/

(continued)

Table 8.2 (continued)

Software	Brief descriptions	Root traits	Licence	Automation level	Plant requirement	Image requirements	Source	Website
RootNav	RootNav facilitates reconstruction and quantification of root architecture. Root data can be exported and then analysed in bulk using the supplied viewer tool	Root length and count; convex hull; insertion and insertion angles	F	Semiautomated	Any	Any	Pound et al. (2013)	sourceforge.net/projects/rootnav/
RootTrak	RootTrak is designed for X-ray CT imaging data. It is able to adapt to changing root density estimates. It can extract a range of root architectural traits for the soil-grown roots	3D reconstruction of roots	F	Semiautomated	Mature, seedlings	Micro-CT image sequence	Mairhofer et al. (2012)	sourceforge.net/projects/roottrak/
RootReader2D	RootReader2D is focusing on high-throughput analysis of total root length and selected root types of interest. The expended package performs lateral root counts for selected roots and has capabilities to work with the width-class groups and branching angles. It has been adapted to work roots systems that are grown in gellan gum, sand, agarose plate and paper pouch growth systems	Root length and depth; number of branches; topological properties	F	Semiautomated	Mature, seedlings	Tiff, jpg, png, bmp	Clark et al. (2013)	www.plantmineralnutrition.net/rootreader.htm

RootReader3D	RootReader3D software is designed to reconstruct and quantify 3D root system architecture descriptors from 2D rotational image sequences	Root length, diameter and surface area; root depth and volume; convex hull; number of branches; root orientation; insertion angles	OD	Automated	Seedlings to mature plants	Projection, any	Clark et al. (2011)	www.plantmineralnutrition.net/rootreader.htm
RootScope	RootScope is a landmark-based allometric method for rapid phenotyping of root system architecture	Root shape	F	Semiautomated	Arabidopsis	gif, tiff, jpg, png	Ristova et al. (2013)	www.atmosstudio.com/Rootscope
RootSnap!	The software has tracing enhancements to snap root tracing points to the centre of the root automatically. It can monitor root growth, disease, dynamics and behaviour over time and simplify mapping roots	Root length, diameter, surface area and volume	C	Semiautomated	Any	Any	Juraniec et al. (2014)	www.cid-inc.com/root-snap
RootTrace	RootTrace allows automatic and high-throughput measure of root length and curvature. It can trace the main root to the tip in every image in a time series. The software has been extended to count emerged lateral roots and to recover strongly curved and agravitropic roots	Root length; curvature; number of branches	O&F	Automated	Arabidopsis, seedlings	Time series	French et al. (2009)	sourceforge.net/projects/roottrace/

(continued)

Table 8.2 (continued)

Software	Brief descriptions	Root traits	Licence	Automation level	Plant requirement	Image requirements	Source	Website
RootView	The package is designed for working with images from minirhizotrons to study the growth and demography of fine roots and mycorrhizas: roots can be classified into different user-defined categories, their length and thickness are automatically measured from tracings, and root tips can be assigned to categories	Root length and diameter	F	Manual	Minirhizotron	jpg	Pedro Jos Aphalo, University of Helsinki	www.my.helsinki.fi/aphalo/RootView.html
Skye	The software captures images at the click of a button from USB 'Plug & Play' video cameras or scanners. It has an automatic calibration function for setting up the pixel size and obtaining more accurate measurements	Root length and surface area; number of branches and root tips	C	Automated	Mature, seedlings	Any	Skye instruments	www.skyeinstruments.com/products/plant-analysis-systems/leaf-area-root-length-systems/
SmartRoot	SmartRoot combines a vectorial representation of root objects with a powerful tracing algorithm which accommodates a wide range of image sources	Root length and diameter; insertion and orientation angles; root branches; topological properties	F	Semiautomated	Seedlings to mature plants	tiff, gif, jpeg, bmp	Lobet et al. (2011)	www.uclouvain.be/en-smartroot

and qualities. It supports a sampling-based analysis of root system images. Requires ImageJ to run

WinRHIZO	WinRHIZO is specifically designed for root measurements, including morphological, topological, architectural and colour analyses. The colour used to draw the root skeleton indicates into which diameter class the part of the root has been classified	Root length, diameter, surface area and volume; topological properties; colour	Automated	Seedlings to mature plants	tif, jpg, png	Arsenault et al. (1995)	www.regentinstruments.com
WinRhizoTRON	The software measures roots and displays complete morphological information on screen. The distribution of root length, area, volume or number of tips is displayed as a function of diameter. It measures the real root diameter distribution rather than the average diameter	Root length, diameter, surface area and volume; topological properties; colour	Manual	Seedlings to mature plants rhizotron	tif, jpg, png	Arsenault et al. (1995)	www.regentinstruments.com

Under 'Licence', *C* commercial, *F* free ware, *O&F* open and free ware, *OD* on demand

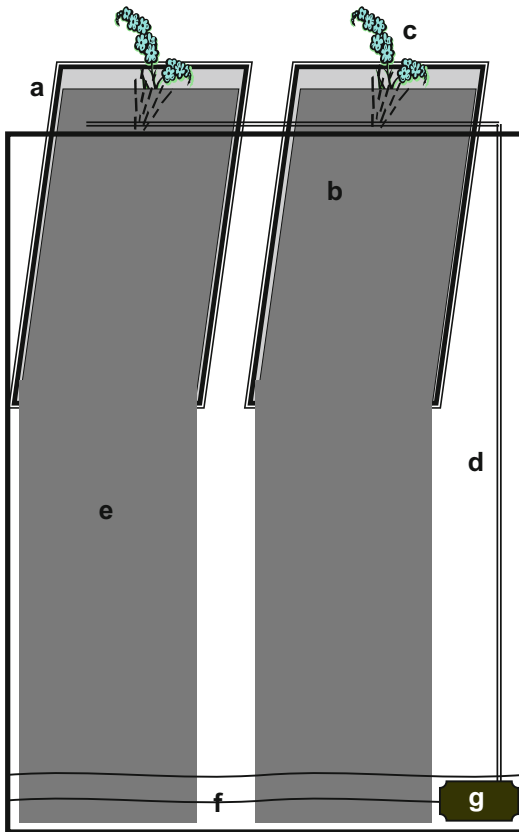


Fig. 8.3 Schematic presentation of the semi-hydroponic phenotyping system for two-dimensional, non-destructive measurements of root system architecture. *A* clear flat acrylic panel, *B* supporting cloth, *C* lupin plant, *D* 240-L bin, *E* extended cloth, *F* water or nutrient solution, *G* pump. Support framework not shown (Modified from Chen et al. 2011)

significantly reduces environmental stresses by optimising water supply with the equipped automatic irrigation system with a controller. Furthermore, it offers the opportunity to expose plants to abiotic stresses (e.g. acidity, salinity, drought, nutrient deficiency, elemental toxicity, etc.) whose evaluation under field conditions is usually quite difficult due to environmental variability affecting the intensity of the stress.

The semi-hydroponic/aeroponic system permits digital mapping of growth dynamics of taproots and lateral roots over time. This growing system overcomes the long-standing unsolved problem of phenotyping large sets of genotypes for rooting traits, which is particularly important

for the identification of QTL and characterisation of molecular markers that may be useful in breeding. The semi-hydroponic phenotyping system was compared with soil-filled pot experiments, and the consistent ranking of root traits was produced for a range of genotypes of narrow-leaved lupin (Chen et al. 2011b).

Growth pouches developed from the hydroponic method were used in characterising root architecture traits such as growth angle and gravitropism of basal roots in common bean (*Phaseolus vulgaris*) related to phosphorus acquisition efficiency (Bonser et al. 1996; Liao et al. 2004). Hund et al. (2009b) improved the pouch system for rapid measurements of lateral root growth of maize. In this system, roots grew on the surface of blotting paper, thus facilitating the two-dimensional observation of root growth over time during the early days of root growth. However, phenotyping large sets of genotypes beyond very early growth stages using pouch systems remains problematic, particularly for QTL mapping studies.

8.3.2 Field

Even though laboratory/glasshouse phenotyping methods provide controlled environments, allow increased throughput and require fewer resources, they may not accurately reflect plant performance under field conditions. Nevertheless, significant associations between root traits of the seedlings grown under controlled conditions and those of the plants grown in the field were found in wheat (Mian et al. 1994; Richards 1996) and maize (Landi et al. 1998; Tuberosa et al. 2002b). However, it is challenging to extrapolate plant performance at the seedling stage when grown in artificial growth media to potential growth in the field (Iyer-Pascuzzi et al. 2010; Sanguineti et al. 2006). Therefore, high-throughput phenotyping in the field is needed to complement and validate studies in the controlled environments.

Field studies provide ground-truthing of plant growth in a particular environment, but phenotyping for root traits in the field conditions

is difficult because of the inability to visualise root systems *in situ*. Traditional methods of observing root system architecture including excavation (followed by root washing) are commonly used in studying roots in the soil environments (Gregory et al. 2003; Smit et al. 2000). Root systems are often manually excavated or augured for quantifying root length, biomass, distribution in soil and morphology and temporal variation of root growth. Excavation-related methods include excavations of (1) root system, (2) undisturbed cores or blocks of soil and (3) installed cores prefilled with root-free soil.

Shovelomics, an emerging term for a high-throughput phenotyping method using root excavation, was recently used for visual scoring of excavated root crowns to assess different root architecture traits of field-grown maize at flowering (Trachsel et al. 2011, 2013). On average, the total time required for excavation, soaking, rinsing and evaluation of root crowns was 10 min for silt-loam and 5 min for sandy soil. The root architectural traits assessed included the number of whorls occupied by brace roots, number of brace roots originating from whorl 1 to whorl 2, the branching density of brace roots and the number, angle and branching density of crown roots. Recently, we used shovelomics technique to evaluate genotypic variability in root traits of eight genotypes of narrow-leafed lupin in response to soil compaction and hardpan in the wheat belt of Western Australia (Chen et al. 2014). Application of shovelomics in other field-grown crop species requires further exploration.

Excavation methods are still commonly used in field studies today simply because of a lack of other reliable techniques. Although excavation techniques can be valuable, they are generally destructive, tedious and time consuming. One of the limitations of excavation-related methods is that they (1) often destroy the topology of the root system, leading to an underestimation of fine roots through breakage during excavation and washing, and (2) make it impossible to evaluate dynamics of root growth (Clark et al. 2011; Smit et al. 2000). Furthermore, root growth and architectural traits in soil environments are inevitably

influenced by (1) soil heterogeneity (Lynch 1995) and (2) physical, chemical and biological interactions in the rhizosphere (Shaff et al. 2009; Ward et al. 2008).

To improve throughput in analysing excavated roots, image analysis methods coupled with data mining approaches have been developed to characterise root architecture. Transparent minirhizotron tubes can be installed vertically, horizontally or at various angles in the field (or in mesocosms) (Bates 1937). Roots that grow around the outside walls of the tubes can be imaged with cameras inserted down the tube length. Minirhizotrons allow the observation of root traits such as elongation rate, density, surface area, number and length at different soil depths throughout the growing season (Ao et al. 2010; Hendrick and Pregitzer 1992; Johnson et al. 2001). Recently, minirhizotrons were used to study root cortical aerenchyma in maize in response to suboptimal availability of soil nutrients, *i.e.* nitrogen, P and potassium (Postma and Lynch 2011), or water deficiency (Zhu et al. 2010). Because repeated observations can be made over time, minirhizotrons are particularly well suited for estimating root production and turnover (Johnson et al. 2001) as well as for estimating root biomass per unit of soil.

One limitation of minirhizotrons is that space may be created around the soil-tube interface that could influence root growth if the tubes are not installed properly. Furthermore, minirhizotrons only capture a fraction of the total root architecture. In this regard, they are better suited for measuring fine roots than coarse roots because fine roots are imaged more frequently and are more likely to be fully captured in images.

Traditional soil coring and trench profiling can be used as complementary techniques to minirhizotrons (Achat et al. 2008; Watt et al. 2005, 2008; Zhu et al. 2010). Like minirhizotrons, however, neither of these methods provides a full description of root system architecture, and both are tedious and time consuming (Vamerali et al. 1999).

A DNA-based method has been established to quantify changes in the root DNA concentration

in soil (Huang et al. 2013; Topp et al. 2013). It may provide fast and accurate measurements of root biomass in soil samples as a complementary method to root quantification.

8.4 Imaging and Analysis Platforms

8.4.1 Imaging Techniques

Root phenotyping approaches discussed above often require efficient imaging techniques, preferably in 3D. Several 3D imaging techniques have recently been used to non-destructively image root systems, including stereoscopies (e.g. Wulfsohn et al. 1999), neutron radiography (Oswald et al. 2008), magnetic resonance imaging (MRI; e.g. Rascher et al. 2011), ground-penetrating radar (e.g. Stover et al. 2007) and X-ray computed tomography (CT; e.g. Flavel et al. 2012).

Stereoscopic methods using plants grown in transparent media (Wulfsohn et al. 1999) enable visualisation of dynamics of root growth using RootViz FS (Phenotype Screening Corporation, Knoxville, TN, USA). Applications of this method are limited due to the need for manually rotating the microscope stage to see different parts of the root system and to image roots horizontally.

Neutron radiography (Oswald et al. 2008), magnetic resonance imaging (Asseng et al. 2000; Rascher et al. 2011), nuclear magnetic resonance (Jahnke et al. 2009; van der Weerd et al. 2001) and ground-penetrating radar (Stover et al. 2007) are used in field studies, but the current scale, resolution, throughput, accessibility and cost efficiency of these techniques limit their utility (Clark et al. 2011). Neutron radiography technique has the advantage in monitoring water distribution and root growth simultaneously, making it suitable for studying root–water relationships in soils (e.g. Oswald et al. 2008; Stingaciu et al. 2013). However, root images produced by neutron radiography are 2D and thus require specific image-analysing software packages (such as Root

System Analyzer, Leitner et al. 2014) to recover root traits. Nuclear magnetic resonance is very sensitive to the type of media used for plant growth. Laser scanning of root systems (Fang et al. 2009) provides precise measurements, but requires relatively long imaging times and can be expensive.

Ground-penetrating radar and electrical resistivity imaging are low-resolution geophysical techniques that have been adapted for non-invasive imaging of roots in field-grown plants. Ground-penetrating radar uses pulses of high-frequency radio waves to image subsurface structures based on differences in their dielectric constants. It is rapid, but detection is generally limited to thick roots (at least 0.5 cm) at relatively shallow depths, depending on the soil type (e.g. dry, sandy soils are optimal). These limitations make ground-penetrating radar primarily useful for measuring root biomass of woody species (Stover et al. 2007).

Recent developments in X-ray CT (micro-scale CT, μ CT) provide a breakthrough technology for non-invasively visualising root growth in soil (Flavel et al. 2012; Garbout et al. 2012; Mooney et al. 2012; Perret et al. 2007; Tray et al. 2010). Even though many papers over the past decades have concentrated on the method development from visualisation of roots in soil (e.g. Gregory et al. 2003) to automated segmentation of the whole root system architecture (e.g. Flavel et al. 2012), some of the most recent research is using X-ray CT to address fundamental questions regarding the functioning of the rhizosphere. Carminati et al. (2009) used X-ray CT to observe the dynamics of air gaps at a 90- μ m resolution in the white lupin rhizosphere in response to wetting and drying cycles. Recent work of Aravena et al. (2013) measured compaction and provided new insights into soil–water uptake in sweet pea (*Lathyrus odoratus*) and sunflower (*Helianthus annuus*) using a 4.4- μ m resolution CT technique.

Sophisticated image processing techniques, frequently based on the object-tracking methods, have demonstrated a great promise in measuring root traits of soil-grown plants at high resolution (Mooney et al. 2012). Using synchrotron

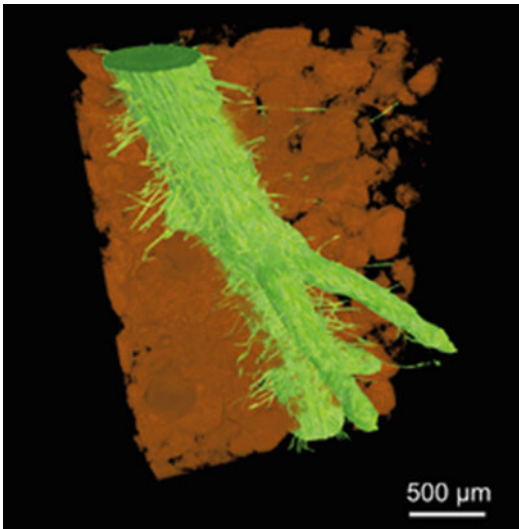


Fig. 8.4 Example image of X-ray computed tomography (CT) showing approx. 3-mm section of a seminal root of wheat (*Triticum aestivum*) (Keyes et al. 2013)

radiation X-ray tomographic microscopy (SRXTM) technique, Keyes et al. (2013) uncovered the 3D interactions of wheat root hairs in soil (Fig. 8.4), leading to the development of a model of phosphate uptake by root hairs based on the geometry of hairs and associated soil pores.

New X-ray CT-based root imaging approaches promise to complement and extend screening for root traits, potentially providing breeders with a ‘deep phenotyping’ capability (Flavel et al. 2012; Mooney et al. 2012). For example, crop root systems could be studied at high resolution and in 3D to reveal which architectural features might be most readily associated with water and nutrient uptake. The quality of ‘region of interest’ scans, i.e. zooming into a large sample and scanning a small volume at high resolution, has also recently improved.

The X-ray CT offers an elegant method of studying root growth non-destructively in situ, but would benefit from substantial hardware and software development to obtain high-resolution images of roots grown in relatively large containers. To that effect, a project recently funded by the European Research Council is

attempting to improve CT hardware, software and genetic selection, offering a potential for enhanced in situ studies of root systems in the future.

8.4.2 Root Image-Analysing Software

High-throughput phenotyping platforms coupled to non-invasive root observation technologies acquire large numbers of root images. More than 30 different software tools are currently available analysing root system images (Table 8.2; Lobet et al. 2013).

General-purpose image-analysing software, such as WinRhizo and ImageJ, may be flexible enough to perform many specialised tasks. Pierret et al. (2013) confirmed the good performance of automated measurement of scanned root images using IJ_Rhizo in comparison with the commercial package WinRhizo.

A number of specifically designed image-analysing packages have also been developed for high-throughput quantification of root architecture traits. For example, there are several software packages for automating the analysis of root traits in minirhizotron images, including RootView, RooTracker, MR-RIPL and WinRhizoTRON. French et al. (2009) described the application of RootTrace software for high-throughput, automatic measurements of *Arabidopsis* seedling roots grown on agarose plates. The method combines a particle-filtering algorithm with a graph-based method to trace the centre line of a root. The package can quantify root length, curvature and stimulus response parameters such as onset of gravitropism, through tracing function. Leitner et al. (2014) developed a novel approach for recovering root architecture traits from 2D neutron radiography images based on image-analysing techniques in Root System Analyzer software. Information about these image-analysing programs and their applications are summarised in Table 8.2. Other useful data are available in Clark et al. (2013) and Lobet et al. (2013).

8.5 Root Models and Simulations

The complex interactions between root systems and their soil environment, and the difficulties associated with visualising and measuring these interactions, make studying the plant–soil continuum a challenge (Dunbabin et al. 2013). Current development of 3D root architectural models offers an excellent opportunity to characterise root function in soil, determine factors governing root–soil interactions and identify root parameters that underpin adaptation to a particular environment. Root models can be used to simulate (1) 3D and time-dynamic root architecture; (2) biological, physical and chemical processes occurring in soil; (3) scenarios beyond those directly observed; and (4) these scenarios in a dynamic environments that vary in time and space (De Dorlodot et al. 2007). Thus, by integrating rhizosphere and growth data, simulation and modelling studies are capable of linking predictive laboratory techniques with field studies, allowing researchers to strategically predict, evaluate and target beneficial root traits or genotypes for specific growth environments (De Dorlodot et al. 2007; Ho et al. 2004).

The development of structure-function root models and the features of six current root models have recently been reviewed in Dunbabin et al. (2013). These six root models are SimRoot (Lynch et al. 1997), SPACSYS (Bingham and Wu 2011; Wu et al. 2007), RootBox (Leitner et al. 2010), ROOTMAP (Diggle 1988a, b; Dunbabin et al. 2002b), RootTyp (Pagès et al. 2004) and R-SWMS (Somma et al. 1997). These models have been used for a wide range of root modelling studies (see Dunbabin et al. 2013).

ROOTMAP and SimRoot, the two simulation models that differ in the structure and functionality of modules, are being used to investigate various root–soil interactions in crops. ROOTMAP model combines the 3D growth and structure of root systems (Diggle 1988a, b) with root responses to spatial and temporal patterns of mineral nitrogen concentration in the

environment (Dunbabin et al. 2001a, b) to produce an interactive model of root structure and function (Dunbabin et al. 2002b). So far, ROOTMAP was used to model (1) root growth of lupins, field peas and wheat and (2) uptake of water and nutrients (N and P) from soils with varying resistances to root growth and differing water and nutrient supplies at scales ranging from rhizosphere (Dunbabin et al. 2006) to field (Dunbabin et al. 2002a). The SimRoot model (Lynch et al. 1997) was previously used to select optimal root traits for phosphorus efficiency in beans, followed by selecting breeding lines and developing commercial cultivars that are now widely grown in Central and South America (Lynch and Brown 2001; Nielsen et al. 1998). Key strengths of SimRoot are (1) sophisticated routines for estimating carbon costs of various root structures and their efficiency in capturing P from soil (Lynch and Ho 2005; Nielsen et al. 1994), (2) use of fractal geometry to estimate 3D root growth from relatively easily measurable root parameters (Nielsen et al. 1997) and (3) capacity to represent changes in physiology and morphology along a root at high spatial resolution. Both models were recently used in studying lupin roots via parameterising with the root data acquired from the semi-hydroponic phenotyping system (Sect. 8.3.1; Chen et al. 2011a). Both models simulated root growth and responses to soil phosphorus in genotypes with contrasting root architecture under growth conditions similar to those of the glasshouse experiment (Chen et al. 2011b, 2013b).

It is anticipated that the structure-function root models will play an increasingly important role in the rhizosphere research, providing insights into the relationships among root architecture, morphology and functional efficiency (Dunbabin et al. 2013). With further development, root models have the potential to be used as an aid in crop breeding programmes by selecting root traits important for enhanced plant performance and grain yield in targeted environments.

8.6 Conclusion

Breeders, agronomists and other researchers recognise the significance of RSA to crop productivity. Increasing attention is being paid to searching for root traits conferring efficiency in resource acquisition and adaptation to edaphic stresses, particularly in drying soil environments. Various technologies are being developed for high-throughput phenotyping, non-invasive visualisation and accurate image analysis of root architecture traits. Together with recent developments of the structure-function simulation models, these advanced approaches will enhance our understanding of the relationship between root architecture and function and the complexity of root–soil interactions, leading to improved crop performance and productivity.

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References

- Achat DL, Trichet P, Bakker MR (2008) Rooting patterns and fine root biomass of *Pinus pinaster* assessed by trench wall and core methods. *J For Res* 13:165–175
- Ao J, Yan X, Liao H, Fu J, Tian J (2010) Genetic variability for root morph–architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. *Funct Plant Biol* 37:304–312
- Araki H, Iijima M (1998) Rooting nodes of deep roots in rice and maize grown in a long tube. *Plant Prod Sci* 1:242–247
- Araki H, Hirayama M, Hirasawa H, Iijima M (2000) Which roots penetrate the deepest in rice and maize root systems? *Plant Prod Sci* 3:281–288
- Aravena JE, Berli M, Menon M, Ghezzehei TA, Mandava AK, Regentova EE, Pillai NS, Steude J, Young MH, Nico PS, Tyler SW (2013) Synchrotron X–ray microtomography – new means to quantify root induced changes of rhizosphere physical properties. In: Anderson SH, Hopmans JW (eds) Soil–water–root processes: advances in tomography and imaging, pp 39–67
- Armengaud P (2009) EZ–Rhizo software. *Plant Signal Behav* 4:139–141
- Armengaud P, Zambaux K, Hills A, Sulpice R, Pattison RJ, Blatt MR, Amtmann A (2009) EZ–Rhizo: integrated software for the fast and accurate measurement of root system architecture. *Plant J* 57:945–956
- Arsenault JL, Poulcur S, Messier C, Guay R (1995) WinRHIZO™, a root–measuring system with a unique overlap correction method. *HortSci* 30:906–906
- Asseng S, Aylmore LAG, MacFall JS, Hopmans JW, Gregory PJ (2000) Computer–assisted tomography and magnetic resonance imaging. In: Smit AL, Bengough AG, Engels C, van Noordwijk M, Pellerin S, van de Geijn SC (eds) Root methods: a handbook. Springer, Berlin/Heidelberg, pp 343–363
- Baddeley JA, Bingham IJ, Hoad SP (2007) Development of cereal root systems for sustainable agriculture. In: Østergård H, Backes G, Kovcs G (eds) Varietal characteristics of cereals in different growing systems with special emphasis on below ground traits. Velence, Hungary, pp 9–13
- Basu P, Pal A (2012) A new tool for analysis of root growth in the spatio–temporal continuum. *New Phytol* 195:264–274
- Basu P, Pal A LJP, Brown KM (2007) A novel image–analysis technique for kinematic study of growth and curvature. *Plant Physiol* 145:305–316
- Bates GH (1937) A device for the observation of root growth in the soil. *Nature* 139:966–967
- Beebe SE, Rojas–Pierce M, Yan X, Blair MW, Pedraza F, Muñoz F, Tohme J, Lynch JP (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. *Crop Sci* 46:413–423
- Bengough AG, Gordon DC, Al–Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant Soil* 262:63–70
- Bingham IJ, Wu L (2011) Simulation of wheat growth using the 3D root architecture model SPACSYS: validation and sensitivity analysis. *Europ J Agron* 34:181–189
- Bonser AM, Lynch J, Snapp S (1996) Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytol* 132:281–288
- Carminati A, Vetterlein D, Weller U, Vogel HJ, Oswald SE (2009) When roots lose contact. *Vadose Zone J* 8:805–809
- Carvalho IS, Ricardo CP, Chaves M (2004) Quality and distribution of assimilates within the whole plant of lupines (*L. albus* and *L. mutabilis*) influenced by water stress. *J Agron Crop Sci* 190:205–210
- Chen YL, Dunbabin VM, Diggle AJ, Siddique KHM, Rengel Z (2011a) Development of a novel semi–hydroponic phenotyping system for studying root architecture. *Funct Plant Biol* 38:355–363
- Chen YL, Dunbabin VM, Postma J, Diggle AJ, Palta JA, Lynch JP, Siddique KHM, Rengel Z (2011b) Phenotypic variability and modelling of root structure of wild *Lupinus angustifolius* genotypes. *Plant Soil* 348:345–364
- Chen YL, Dunbabin VM, Diggle AJ, Siddique KHM, Rengel Z (2012) Assessing variability in root parameters of wild *Lupinus angustifolius* germplasm: basis for modelling root system structure. *Plant Soil* 354:141–155
- Chen YL, Dunbabin VM, Diggle AJ, Siddique KHM, Rengel Z (2013a) Phosphorus starvation boosts carboxylate secretion in P–deficient genotypes of *Lupinus*

- angustifolius* with contrasting root structure. *Crop Pasture Sci* 64:588–599
- Chen YL, Dunbabin VM, Postma JA, Diggle AJ, Siddique KHM, Rengel Z (2013b) Modelling root plasticity and response of narrow-leaved lupin to heterogeneous phosphorus supply. *Plant Soil* 372:319–337
- Chen YL, Palta J, Clements J, Buirchell B, Siddique KHM, Rengel Z (2014) Root architecture alteration of narrow-leaved lupin and wheat in response to soil compaction. *Field Crops Res* 165:61–70
- Clark LJ, Whalley WR, Leigh RA, Dexter AR, Barraclough PB (1999) Evaluation of agar and agarose gels for studying mechanical impedance in rice roots. *Plant Soil* 207:37–43
- Clark RT, MacCurdy RB, Jung JK, Shaff JE, McCouch SR, Aneshansley DJ, Kochian LV (2011) Three-dimensional root phenotyping with a novel imaging and software platform. *Plant Physiol* 156:455–465
- Clark RT, Famoso AN, Zhao K, Shaff JE, Craft EJ, Bustamante CD, McCouch SR, Aneshansley DJ, Kochian LV (2013) High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. *Plant Cell Environ* 36:454–466
- De Dorlodot S, Forster B, Pages L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends Plant Sci* 12:474–481
- Diggle AJ (1988a) ROOTMAP – a model in three-dimensional coordinates of the growth and structure of fibrous root systems. *Plant Soil* 105:169–178
- Diggle AJ (1988b) ROOTMAP: a root growth model. *Maths Comp Simul* 30:175–180
- Doussan C, Pages L, Pierret A (2003) Soil exploration and resource acquisition by plant roots: an architectural and modelling point of view. *Agronomie* 23:419–431
- Dubrovsky JG, Gambetta GA, Hernandez-Barrera A, Shishkova S, Gonzalez I (2006) Lateral root initiation in *Arabidopsis*: developmental window, spatial patterning, density and predictability. *Ann Bot* 97:903–915
- Dunbabin V, Rengel Z, Diggle A (2001a) *Lupinus angustifolius* has a plastic uptake response to heterogeneously supplied nitrate while *Lupinus pilosus* does not. *Aust J Agr Res* 52:505–512
- Dunbabin V, Rengel Z, Diggle A (2001b) The root growth response to heterogeneous nitrate supply differs for *Lupinus angustifolius* and *Lupinus pilosus*. *Aust J Agr Res* 52:495–503
- Dunbabin V, Rengel Z, Diggle A (2002a) Simulation of field data by a basic three-dimensional model of interactive root growth. *Plant Soil* 239:39–54
- Dunbabin V, Rengel Z, Diggle A, van Hugten R (2002b) Modelling the interactions between water and nutrient uptake and root growth. *Plant Soil* 239:19–38
- Dunbabin VM, McDermott S, Bengough AG (2006) Upscaling from rhizosphere to whole root system: modelling the effects of phospholipid surfactants on water and nutrient uptake. *Plant Soil* 283:57–72
- Dunbabin VM, Postma JA, Schnepf A, Pagès L, Javaux M, Wu L, Leitner D, Chen YL, Rengel Z, Diggle AJ (2013) Modelling root–soil interactions using three-dimensional models of root growth, architecture and function. *Plant Soil* 372:93–124
- Eshel A, Beeckman T (2013) *Plant roots: the hidden half*. CRC Press, New York
- Fang S, Yan X, Liao H (2009) 3D reconstruction and dynamic modeling of root architecture in situ and its application to crop phosphorus research. *Plant J* 60:1096–1108
- Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. *Ann Rev Plant Biol* 64:267–291
- Fitter A, Williamson L, Linkohr B, Leyser O (2002) Root system architecture determines fitness in an *Arabidopsis* mutant in competition for immobile phosphate ions but not for nitrate ions. *Proc Roy Soc B–BiolSci* 269:2017–2022
- Flavel RJ, GuppyCN TM, Watt M, McNeill A, Young IM (2012) Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography. *J Exp Bot* 63:2503–2511
- French A, Ubeda-Toms S, Holman TJ, Bennett MJ, Pridmore T (2009) High-throughput quantification of root growth using a novel image-analysis tool. *Plant Physiol* 150:1784–1795
- Futsaether CM, Oxaal U (2002) A growth chamber for idealized studies of seedling root growth dynamics and structure. *Plant Soil* 246:221–230
- Galkovskyi T, Mileyka Y, Bucksch A, Moore B, Symonova O, Price CA, ToppCN I-PAS, Zurek PR, Fang S, Harer J, Benfey PN, Weitz JS (2012) GiA Roots: software for the high throughput analysis of plant root system architecture. *BMC Plant Biol* 12:116
- Garbout A, Munkholm LJ, HansenSB PBM, Munk OL, Pajor R (2012) The use of PET/CT scanning technique for 3D visualization and quantification of real-time soil/plant interactions. *Plant Soil* 352:113–127
- Gregory PJ (2008) *Plant roots: growth, activity and interactions with the soil*. Wiley, Oxford
- Gregory PJ, Hutchison DJ, Read DB, Jenneson PM, Gilboy WB, Morton EJ (2003) Non-invasive imaging of roots with high resolution X-ray micro-tomography. *Plant Soil* 255:351–359
- Gregory PJ, Hutchison DJ, Read DB, Jenneson PM, Gilboy WB, Morton EJ (2003) Non-invasive imaging of roots with high resolution X-ray micro-tomography. *Plant Soil* 255:351–359
- Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young M (2009) Root phenomics of crops: opportunities and challenges. *Funct Plant Biol* 36:922–929
- Hall DJM, Jones HR, Crabtree WL, Daniels TL (2010) Claying and deep ripping can increase crop yields and profits on water repellent sands with marginal fertility in southern Western Australia. *Soil Res* 48:178–187

- Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. *J Exp Bot* 60:1953–1968
- Hargreaves CE, Gregory PJ, Bengough AG (2009) Measuring root traits in barley (*Hordeum vulgare* ssp. *vulgare* and ssp. *spontaneum*) seedlings using gel chambers, soil sacs and X-ray microtomography. *Plant Soil* 316:285–297
- Hendrick RL, Pregitzer KS (1992) Spatial variation in tree root distribution and growth associated with minirhizotrons. *Plant Soil* 143:283–288
- Hinsinger P, Brauman A, DevauN GF, Jourdan C, Laclau JP, Le Cadre E, Jaillard B, Plassard C (2011) Acquisition of phosphorus and other poorly mobile nutrients by roots: where do plant nutrition models fail? *Plant Soil* 348:29–61
- Ho MD, McCannon BC, Lynch JP (2004) Optimization modeling of plant root architecture for water and phosphorus acquisition. *J Theo Biol* 226:331–340
- Ho MD, Rosas JC, Brown KM, Lynch JP (2005) Root architectural tradeoffs for water and phosphorus acquisition. *Funct Plant Biol* 32:737–748
- Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol* 162:9–24
- Huang CY, Kuchel H, Edwards J, Hall S, Parent B, Eckermann P, Herdina HDM, Langridge P, McKay AC (2013) A DNA-based method for studying root responses to drought in field-grown wheat genotypes. *SciRep*3. doi:10.1038/srep03194
- Hund A, Ruta N, Liedgens M (2009a) Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. *Plant Soil* 318:311–325
- Hund A, Trachsel S, Stamp P (2009b) Growth of axile and lateral roots of maize: I. Development of a phenotyping platform. *Plant Soil* 325:335–349
- Iyer–Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Weitz JS, Benfey PN (2010) Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiol* 152:1148–1157
- Jahnke S, Menzel MI, Van Dusschoten D, Roeb GW, Bühler J, Minwuyelet S, Blümmler P, Temperton VM, Hombach T, Streun M, Beer S, Khodaverdi M, Ziemons K, Coenen HH, Schurr U (2009) Combined MRI–PET dissects dynamic changes in plant structures and functions. *Plant J* 59:634–644
- Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. *Environ Exp Bot* 45:263–289
- Juranić M, Lequeux H, Hermans C, Willems G, Nordborg M, Schneeberger K, Salis P, Vromant M, Lutts S, Verbruggen N (2014) Towards the discovery of novel genetic component involved in stress resistance in *Arabidopsis thaliana*. *New Phytol* 201:810–824
- Kamoshita A, Wade LJ, Yamauchi A (2000) Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during the drought period. *Plant Prod Sci* 3:189–196
- Keyes SD, Daly KR, Gostling NJ, Jones DL, Talboys P, Pinzer BR, Boardman R, Sinclair I, Marchant A, Roose T (2013) High resolution synchrotron imaging of wheat root hairs growing in soil and image based modelling of phosphate uptake. *New Phytol* 198:1023–1029
- Kimura K, Kikuchi S, Yamasaki SI (1999) Accurate root length measurement by image analysis. *Plant Soil* 216 (1–2):117–127
- Kondo M, Murty MV, Aragonés DV, Okada K, Winn T, Kwak KS (1999) Characteristics of the root system and water uptake in upland rice. In: Ito O, O’Toole J, Hardy B (eds) Genetic improvement of rice for water limited environments. International Rice Research Institute, Makati City, pp 117–131
- Lambers H, Brundrett MC, Raven JA, Hopper SD (2011) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348:7–27
- Landi P, Giuliani MM, Sanguineti MC, Albrecht B (1998) Seedling characteristics in hydroponic culture and field performance of maize genotypes with different resistance to root lodging. *Maydica* 43:111–116
- Le Bot J, Serra V, Fabre J, Draye X, Adamowicz S, Pagès L (2010) DART: a software to analyse root system architecture and development from captured images. *Plant Soil* 326:261–273
- Leitner D, Klepsch S, Knieß A, Schnepf A (2010) The algorithmic beauty of plant roots – an L-system model for dynamic root growth simulation. *Math Comp Model Dyn* 16:575–587
- Leitner D, Felderer B, Vontobel P, Schnepf A (2014) Recovering root system traits using image analysis exemplified by two-dimensional neutron radiography images of lupine. *Plant Physiol* 164:24–35
- Liao H, Yan XL, Rubio G, Beebe SE, Blair MW, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Funct Plant Biol* 31:959–970
- Liu X, Zhang S, Shan L, Yang X, Wu A (2007) The heterosis of water uptake by single root of maize. *Acta Agron Sin* 33:1625–1629
- Lobet G, Pagès L, Draye X (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiol* 157:29–39
- Lobet G, Draye X, Prilleux C (2013) An online database for plant image analysis software tools. *Plant Methods* 9:38
- Lynch J (1995) Root architecture and plant productivity. *Plant Physiol* 109:7–13
- Lynch JP (2007) Roots of the second green revolution. *Aus J Bot* 55:493–512
- Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. *Philos Trans R Soc B* 367:1598–1604

- Lynch JP, Brown KM (2001) Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. *Plant Soil* 237:225–237
- Lynch JP, Ho MD (2005) Rhizoeconomics: carbon costs of phosphorus acquisition. *Plant Soil* 269:45–56
- Lynch JP, Nielsen KL, Davis RD, Jablonski AG (1997) SimRoot: modelling and visualization of root systems. *Plant Soil* 188:139–151
- Mairhofer S, Zappala S, Tracy SR, Sturrock C, Bennett M, Mooney SJ, Pridmore T (2012) RooTrak: automated recovery of three-dimensional plant root architecture in soil from X-ray microcomputed tomography images using visual tracking. *Plant Physiol* 158:561–569
- Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ* 28:67–77
- Manschadi AM, Manske GGB (2013) Root architecture and resource acquisition: wheat as a model plant. In: Eshel A, Beeckman T (eds) *Plant roots—the hidden half*, 4th edn. CRC Press, London
- Manschadi AM, Christopher J, Voil PD, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. *Funct Plant Biol* 33:823–837
- Manschadi AM, Hammer GL, Christopher JT, deVoil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). *Plant Soil* 303:115–129
- Manschadi AM, Christopher JT, Hammer GL, Devoil P (2010) Experimental and modelling studies of drought-adaptive root architectural traits in wheat (*Triticum aestivum* L.). *Plant Biosyst* 144:458–462
- Mian MAR, Nafziger ED, Kolb FL, Teyker RH (1994) Root size and distribution of fieldgrown wheat genotypes. *Crop Sci* 34:810–812
- Mooney SJ, Pridmore TP, Helliwell J, Bennett MJ (2012) Developing X-ray Computed Tomography to non-invasively image 3-D root systems architecture in soil. *Plant Soil* 352:1–22
- Nagel KA, Kastenholz B, Jahnke S, Van Dusschoten D, Aach T, Mühlich M, Truhnd Scharr H, Terjung S, Walter A, Schurr U (2009) Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. *Funct Plant Biol* 36:947–959
- Nielsen KL, Lynch JP, Jablonski AG, Curtis PS (1994) Carbon cost of root systems: an architectural approach. *Plant Soil* 165:161–169
- Nielsen KL, Lynch JP, Weiss HN (1997) Fractal geometry of bean root systems: correlations between spatial and fractal dimension. *Am J Bot* 84:26–33
- Nielsen KL, Miller CR, Beck D, Lynch JP (1998) Fractal geometry of root systems: field observations of contrasting genotypes of common bean (*Phaseolus vulgaris* L.) grown under different phosphorus regimes. *Plant Soil* 206:1998–1999
- Nielsen KL, Eshel A, Lynch JP (2001) The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. *J Exp Bot* 52:329–339
- Ober ES, Clark CJ, Bloa ML, Smith CH (2005) Root growth, soil water extraction and drought tolerance in sugar beet. *Aspects Appl Biol* 73:213–220
- Oswald S, Menon M, Carminati A, Vontobel P, Lehmann E, Schulin R (2008) Quantitative imaging of infiltration, root growth, and root water uptake via neutron radiography. *Vadose Zone J* 7:1035–1047
- Pagès L, Vercambre G, Drouet JL, Lecompte F, Collet C, Le Bot J (2004) Root Typ: a generic model to depict and analyse the root system architecture. *Plant Soil* 258:103–119
- Paterson E, Sim A, Standing D, Dorward M, McDonald AJS (2006) Root exudation from *Hordeum vulgare* in response to localized nitrate supply. *J Exp Bot* 57:2413–2420
- Pearse SJ, Veneklaas EJ, Cawthray GR, Bolland MD, Lambers H (2006) Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant Soil* 288:127–139
- Perret JS, Al-Belushi ME, Deadman M (2007) Non-destructive visualization and quantification of roots using computed tomography. *Soil Biol Biochem* 39:391–399
- Pierret A, Doussan C, Pages L (2006) Spatio-temporal variations in axial conductance of primary and first-order lateral roots of a maize crop as predicted by a model of the hydraulic architecture of root systems. *Plant Soil* 282:117–126
- Pierret A, Gonkhamdee S, Jourdan C, Maeght JL (2013) IJ_Rhizo: an open-source software to measure scanned images of root samples. *Plant Soil* 373:531–539
- Postma JA, Lynch JP (2011) Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiol* 156:1190–1201
- Pound MP, French AP, Atkinson JA WDM, Bennett MJ, Pridmore T (2013) RootNav: navigating images of complex root architectures. *Plant Physiol* 162:1802–1814
- Rascher U, Blossfelds FF, Jahnke S, Jansen M, Kuhn AJ, Matsubara S, Martin LLA, Merchant A, Metzner R, Müller-Linow M, Nagel KA, Pieruschka R, Pinto F, Schreiber CM, Temperton VM, Thorpe MR, van Dusschoten D, van Volkenburgh E, Windt CW, Schurr U (2011) Non-invasive approaches for phenotyping of enhanced performance traits in bean. *Funct Plant Biol* 38:968–983
- Rengel Z (2005) Breeding crops for adaptation to environments with low nutrient availability. In: Ashraf M, Harris PJC (eds) *Abiotic stresses: plant resistance through breeding and molecular approaches*. The Haworth Press, New York, pp 239–276
- Rengel Z (2013) *Improving water and nutrient-use efficiency in food production systems*. Wiley-Blackwell, Ames
- Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant Growth Regul* 20:157–166
- Ristova D, Rosas U, Krouk G, Ruffel S, Birnbaum KD, Coruzzi GM (2013) RootScape: a landmark-based

- system for rapid screening of root architecture in Arabidopsis. *Plant Physiol* 161:1086–1096
- Sanguineti MC, Duvick DN, Smith S, Landi P, Tuberosa R (2006) Effects of long-term selection on seedling traits and ABA accumulation in commercial maize hybrids. *Maydica* 51:329–338
- Shaff J, Schultz B, Craft E, Clark R, Kochian L (2009) GEOCHEM-EZ: a chemical speciation program with greater power and flexibility. *Plant Soil* 303:207–214
- Shi L, Shi T, Broadley MR, White PJ, Long Y, Meng J, Xu F, Hammond JP (2013) High-throughput root phenotyping screens identify genetic loci associated with root architectural traits in *Brassica napus* under contrasting phosphate availabilities. *Ann Bot* 112:381–389
- Singh D, Dikshit HK, Singh R (2013) A new phenotyping technique for screening for drought tolerance in lentil (*Lens culinaris* Medik.). *Plant Breed* 132:185–190
- Smit AL, Bengough AG, Engels C, van Noordwijk M, Pellerin S, van de Geijn SC (2000) Root methods: a handbook. Springer, Berlin
- Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. *Phil Trans Royal Society B: Biol Sci* 367:1441–1452
- Somma F, Clausnitzer V, Hopmans JW (1997) An algorithm for three-dimensional, simultaneous modeling of root growth, transient soil water flow, and solute transport and uptake. Version 2.1. Paper No. 100034. Dept of Land, Air, and Water Resources, University of California
- Steele KA, Price AH, Shashidhar HE, Witcombe JR (2006) Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor Appl Gen* 112:208–221
- Stingaciu L, Schulz H, Pohlmeier A, Behnke S, Zilken H, Javaux M, Vereecken H (2013) In situ root system architecture extraction from magnetic resonance imaging for application to water uptake modeling. *Vadose Zone J* 12:1–9
- Stover DB, Drake BG, Butnor JR, Day FP (2007) Effect of elevated CO₂ on coarse-root biomass in Florida scrub detected by ground-penetrating radar. *Ecology* 88:1328–1334
- Struik PC, Yin X (2007) Combined model and QTL analysis of crop physiological traits in barley. In: Østergård H, Backes G, Kovcs G (eds) Varietal characteristics of cereals in different growing systems with special emphasis on below ground traits. Velence, Hungary, pp 25–30
- Tajima R, Kato Y (2011) Comparison of threshold algorithms for automatic image processing of rice roots using freeware. *Image J Field Crops Res* 121:460–463
- Topp CN, Iyer-Pascuzzi AS, Anderson JT, Lee CR, Zurek PR, Symonova O, Zheng Y, Bucksch A, Mileyko Y, Galkovskiy T, Moore BT, Harer J, Edelsbrunner H, Mitchell-Olds T, Weitz JS, Benfey PN (2013) 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. *PNAS* 110: E1695–E1704
- Trachsel S, Kaeppler S, Brown K, Lynch J (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* 341:75–87
- Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2013) Maize root growth angles become steeper under low N conditions. *Field Crop Res* 140:18–31
- Tracy SR, Roberts JA, BlackCR MNA, Davidson R, Mooney SJ (2010) The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. *J Exp Bot* 61:311–313
- Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S (2002a) Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Ann Bot* 89:941–963
- Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002b) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Mol Biol* 48:697–712
- Valizadeh GR, Rengel Z, Rate AW (2003) Response of wheat genotypes efficient in P utilisation and genotypes responsive to P fertilisation to different P banding depths and watering regimes. *Aust J Agric Res* 54:59–65
- Vamerali T, Ganis A, Bona S, Mosca G (1999) An approach to minirhizotron root image analysis. *Plant Soil* 217:183–193
- van der Weele CM, Jiang HS, Palaniappan KK, Ivanov VB, Palaniappan K, Baskin TI (2003) A new algorithm for computational image analysis of deformable motion at high spatial and temporal resolution applied to root growth. Roughly uniform elongation in the meristem and also, after an abrupt acceleration, in the elongation zone. *Plant Physiol* 132:1138–1148
- van der Weerd L, Claessens MM, RuttinkT VFJ, Schaafsma TJ, Van As H (2001) Quantitative NMR microscopy of osmotic stress responses in maize and pearl millet. *J Exp Bot* 52:2333–2343
- Wang E, Smith CJ (2004) Modelling the growth and water uptake function of plant root systems: a review. *Aust J Agric Res* 55:501–523
- Watt M, Rebetzke GJ, Kirkegaard JA (2005) A wheat genotype developed for rapid leaf growth copes well with the physical and biological constraints of unploughed soil. *Funct Plant Biol* 32:695–706
- Watt M, Silk WK, Passioura JB (2006) Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Ann Bot (Lond)* 97:839–855
- Watt M, Magee LJ, McCully ME (2008) Types, structure and potential for axial water flow in the deepest roots of field-grown cereals. *New Phytol* 178:135–146
- Wiese AH, Riemenschneider DE, Zalesny RS (2005) An inexpensive rhizotron design for two-dimensional,

- horizontal root growth measurements. *Tree Planters Notes* 51:40
- Wong MTF, Asseng S (2006) Determining the causes of spatial and temporal variability of wheat yields at sub-field scale using a new method of upscaling a crop model. *Plant Soil* 283:203–215
- Wu L, McGechan MB, Watson CA, Baddeley JA (2005) Developing existing plant root system architecture models to meet future agricultural challenges. *Adv Agron* 85:181–219
- Wu L, McGechan MB, McRoberts N, Baddeley JA, Watson CA (2007) SPACSYS: integration of a 3D root architecture component to carbon, nitrogen and water cycling – Model description. *Ecol Model* 200:343–359
- Wulfsohn D, Nyengaard JR, Gundersen HJG, Cutler AJ, Squires TM (1999) Non-destructive, stereological estimation of plant root lengths, branching pattern and diameter distribution. *Plant Soil* 214:15–26
- Zeng G, Birchfield ST, Wells CE (2008) Rapid automated detection of roots in minirhizotron images. *Mach Vis Appl* 21:309–317
- Zhu JM, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant Cell Environ* 33:740–749
- Zhu J, Ingram AP, Benfey NP, Elich T (2011) From lab to field, new approaches to phenotyping root system architecture. *Cur Opin Plant Biol* 14:310–317