Physiol Mol Biol Plants (June 2022) 28(6):1311–1321
https://doi.org/10.1007/s12298-022-01209-0

REVIEW ARTICLE

Recent trends in root phenomics of plant systems with available methods- discrepancies and consonances

Mohammad Urfan[1] · Shubham Sharma[1] · Haroon Rashid Hakla[1] · Prakriti Rajput[1] · Sonali Andotra[1] · Praveen K. Lehana[2] · Renu Bhardwaj[3] · M. Suhail Khan[4] · Ranjan Das[5] · Sunil Kumar[6] · Sikander Pal[1]

Received: 9 October 2021 / Revised: 2 July 2022 / Accepted: 12 July 2022 / Published online: 20 July 2022

Abstract The phenotyping of plant roots is a challenging task and poses a major lacuna in plant root research. Roots rhizospheric zone is affected by several environmental cues among which salinity, drought, heavy metal and soil pH are key players. Among biological factors, fungal, nematode and bacterial interactions with roots are vital for improving nutrient uptake efficiency in plants. The subterranean nature of a plant root and the limited number of approaches for root phenotyping offers a great challenge to the plant breeders to select a desirable root trait under different stress conditions. Identification of key root traits can provide a basic understanding for generating crop plants with enhanced ability to withstand various biotic or abiotic stresses. For instance, crops with improved soil exploration potential, phosphate uptake efficiency, water use efficiency and others. Laboratory methods such as hydroponics, rhizotron, rhizoslide and luminescence observatory for roots do not provide precise and desired root quantification attributes. Though 3D imaging by X-ray computed tomography (X-ray-CT) and magnetic resonance imaging techniques are complex, however, it provides the most applicable and practically relevant data for quantifying root system architecture traits. This review outlines the current developments in root studies including recent approaches viz. X-ray-CT, MRI, thermal infrared imaging and minirhizotron. Although root phenotyping is a laborious procedure, it offers multiple advantages by removing discrepancies and providing the actual practical significance of plant roots for breeding programs.

✉ Sikander Pal
sikanderpal@jammuuniversity.ac.in;
sikanderchowdhary@gmail.com

Mohammad Urfan
urfanbutt1992@gmail.com

Shubham Sharma
shubhamsharma199425@gmail.com

Haroon Rashid Hakla
haroonhakla@rediffmail.com

Prakriti Rajput
prakritirajput2512@gmail.com

Sonali Andotra
sonaliandotra22@gmail.com

Praveen K. Lehana
pklehana@gmail.com

Renu Bhardwaj
renubhardwaj82@gmail.com

M. Suhail Khan
drkhansuhail@gmail.com

Ranjan Das
rdassam1966@gmail.com

Sunil Kumar
sunilbhougal06@gmail.com

1 Plant Physiology Laboratory, Department of Botany, University of Jammu, Jammu 180006, India

2 Department of Physics, University of Jammu, Jammu 180006, India

3 Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143001, India

4 USBT, Guru Gobind Singh Indraprastha University, Dwarka 110 078, New Delhi, India

5 Department of Crop Physiology, Assam Agricultural University, Jorhat 785013, India

6 Department of Statistics, University of Jammu, Jammu 180006, India

Keywords Plant roots · Non-invasive · Phenotyping · Rhizotrons · Root system architecture

Introduction

Plant roots are involved in the absorption of water and nutrients, anchorage, growth and development, food storage, and as interacting sites for many biotic communities. In response to the changing water and nutrient profiles in the soil, the dynamics of plant root growth and development serve as a black box for exploring the natural variation and identifying the essential root traits to improve plant productivity (Lynch 1995; Kano et al 2011; Pal et al. 2017; Hakla et al. 2021; Sharma et al. 2021; Urfan et al. 2021). In most the cereal crops, the root structure is fundamentally of two types viz. embryonic roots-primary root (PRs) and seminal root (SRs) which develop directly from embryo and post-embryonic roots-crown roots (CRs) and brace root (BRs). Both of these root systems are collectively known as adventitious roots which develop from the shoot node (Hochholdinger 2009; Lynch and Brown 2012). The growth and development (length, number, surface area, angle, lateral root density, and longevity) of all the root types are highly regulated and governed by genetic and environmental factors independently or through interaction with each other. In a specific environmental condition, the pattern and distribution of a root system are known as root system architecture (RSA). The RSA being vibrant is influenced by a variety of factors such as soil water status, temperature fluctuation, nutrients status, and pH (Bao et al. 2014). The nature of the RSA facilitates plants to reply, acclimatize and flourish in ever-changing environmental conditions. However, selecting crops based on important RSA traits poses difficult practical challenges. The role of root traits under different environmental conditions has been correlated with crop productivity in rice and spring wheat (Kell 2011; Uga et al. 2013; Narayanan et al. 2014). The relationship between root traits with agricultural yield under different biological and environmental stresses has been studied extensively (Mahanta et al. 2014; Le Marié et al. 2014; Mathieu et al. 2015; Tiziano et al. 2021).

Methods based on captured images (scanners or cameras) are mostly applied for computing the morphometric traits of root and shoot. These procedures permit several phenotypes in a short span of time (Clark et al. 2013; Adu et al. 2014; Le Marié et al. 2014). Furthermore, the 3D imaging technique of RSA can be done with X-ray computed tomography (X-ray-CT), magnetic resonance imaging (MRI), or neutron tomography (NT) (Leitner et al. 2014; Metzner et al. 2015). The hydroponics approach, in which plants are grown without soil, preferably facilitates the root growth observation (Conn et al. 2013; Mathieu et al. 2015). This method is easily applied, replicated, and generally economical, which provides significant benefits. Another non-destructive method is Rhizotron systems, consisting of concealed rooms, laboratories, or plane containers with clear glass or plastic windows to expose the soil for root visualization. Rhizotrons are widely used to observe the root growth and developmental changes of a large number of plants in a soil-like substrate (Chen et al. 2014) and allow a fine analysis of soil-root relationships.

This review describes different approaches to studying the RSA. It will attempt to establish a greater clarity of current knowledge about technologies used to study plant RSA. The main highlights of this article include an overview of the methodologies and the approaches for plant root studies such as X-ray computed tomography (X-ray CT), magnetic resonance imaging (MRI), thermal infrared imaging, and minirhizotron (MR) with their advantages, disadvantages, discrepancies and practical significances for crop and forage breeding programs.

Root phenotyping strategies

One of the most important and necessary parameters considered for the in-depth understanding of the growth and development of plants under various biotic and abiotic stresses is the precise measurement of a root system. The RSA is studied mostly through laboratory-based methods and in field conditions. Environmental conditions are highly controlled and regulated in laboratory methods, thus providing real-time analysis for RSA. The early RSA traits have been thoroughly examined and estimated under different environmental stimuli. In order to gain knowledge of the architectural complexity of roots, for researchers to be able to observe root growth kinetics and its developmental pattern is extremely important. The transparent nature of the media, in which roots become opaque, offers great difficulty in estimating RSA traits quantitatively. Various soil-less strategies, including aeroponics, in which roots of the desired plant are hung in the air and sprayed with a fine mist of nutrient solutions (Gangopadhyay et al. 2021) and hydroponics (Fig. 1a) in which plants are grown in containers (static hydroponics) and PVC pipes (water circulating hydroponics) filled with water and nutrients are in practice for RSA studies (Tiziano et al. 2021). The paper roll method (Fig. 1b), which is equally competent and an easy approach to analyzing and recording early RSA traits, under different environmental cues is also widely used in wheat and mung bean seedlings respectively (Alemu et al. 2021; Hakla et al. 2021). These methods offer minimal physical resistance to the plant root system growth due to that its 3- dimensional shape, thus RSA studies in lab-based methods will not infer the true or actual response of RSA under field conditions. In addition

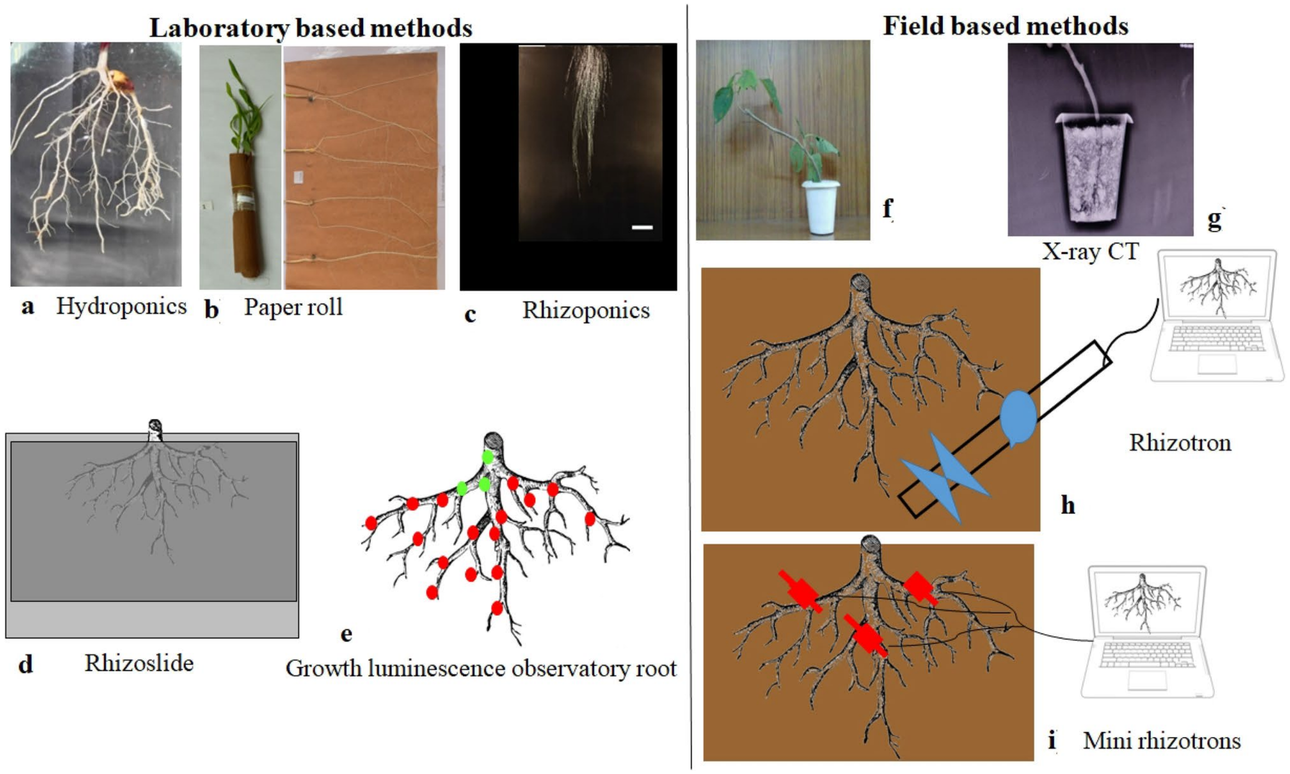

Fig. 1 Root system analyzing methods in use under laboratory and field conditions. Laboratory based methods: **a** hydroponics, **b** paper roll, **c** rhizoponics, **d** rhizoslide (for details Le Marié et al. 2014), **f** growth luminescence observatory root (for details Rellán-Álvarez et al. 2015) Field based methods: **f** plant in pot filled with soil, **g** X-ray of plant roots in soil pot conditions (for details Lontoc-Roy et al. (2006), **h** rhizotron (for details Rahman et al. 2020), **i** (for details) minirhizotrons method (for details Rahman et al. 2020)

to these methods, novel techniques and methodologies used in laboratory and greenhouse conditions for RSA analysis were thoroughly investigated and compared for advantages and their discrepancies.

Rhizoponics

The hydroponic rhizotrons are used for analyzing the RSA of mature *Arabidopsis* plants. In hydroponics plants are grown without soil and the nutrients are regulated manually, and their influences on RSA can be explored in-depth (Fig. 1c). Interestingly, rhizoponics is the combination of hydroponics and rhizotron, which enables a researcher to study RSA in *Arabidopsis* and other similar plants.

The rhizoponics method provides precise quantification of root development in plants. Important root traits such as root surface area, length, depth, width and lateral root density are measurable with rhizoponic setup in *Arabidopsis* plants (Mathieu et al. 2015). This setup has been used to characterize the RSA and shoot growth from seedling to adult stages, i.e., from seed to seed in *Arabidopsis*. The system thus offers the advantages of hydroponics in controlling the root environment with easy access to the roots for measurements of key root attributes.

Rhizoslide

Rhizoslide involves the growing of a plant with-in a layer of two-dimensional large plates (Fig. 1d). Its central glass shelter stabilizes the root system and is covered on mutual sides with germination sheets, which provide substrate, water and nutrient for the developing embryo. Primary root and seminal roots grow hidden between a plexiglass surface and germination sheets; however, crown roots growth could be visibly detected. Rhizoslide could create a plant growth system that enables non destructive measurement of the RSA. The platform distinguishes easily between embryonic and post-embryonic roots and is useful for the genetic studies of crown roots and other root types which are adaptive in character for the management of abiotic and biotic stresses. Similarly, it offers a great opportunity in studying the role of nutrients in root system development. Several studies have been carried out using rhizoslide on both dicot and monocot species (For more details, Le et al. 2014; Perelman et al. 2020). Estimation of seminal roots and screening of quantitative trait loci in wheat under different environmental factors (Boudiar et al. 2020).

Transparent pots

The use of a transparent or clear pot is an efficient and economical root phenotyping method to study desirable RSA traits. Seeds are placed vertically, embryos downward along the wall of the pot. After sowing, the transparent pots are placed in the black pots in order to protect against the influence of light on root growth. For root image acquisition of primary roots and seminal roots, the camera is placed on the tripod strand and there after images are taken or recorded from different angles by rotating the pot. Transparent pots have been successfully used for high-throughput system analysis of RSA in wheat seedlings (Richard et al. 2015). This method is used for measuring lateral root density, root behavior and their distribution in correlation with the natural environment in plants (Neumann et al. 2009) (Table 2).

Growth luminescence observatory

The growth luminescence observatory (GLO) is a plant root imaging platform for the analysis of RSA (Fig. 1e) (Rellán-Álvarez et al. 2015). In GLO, luminescent markers are used, which enable the researchers to examine and investigate the changes occurring during root growth and development *via* visualizing the root system of a plant grown in thin and soil-filled transparent pots (Rellán-Álvarez et al. 2015; LaRue 2020). Experimental set up and use of GLO is explicitly presented in Rellán-Álvarez et al. (2015). The GLO-ROOT, allow the researchers to study RSA traits such as root length, the direction of roots, their shape and root depth as well as their gene expression. GLO-ROOT can be used for the measurement of soil water content in soil at different levels, while studying the water status impact on the RSA. Its applications are well established in many plant species such as *Arabidopsis* and *Brachypodium distachyon* in analyzing the RSA (Rellán-Álvarez et al. 2015). Further, it is extended widely for a better understanding of RSA response under low water conditions, phosphate deficiency and light. Furthermore, GLO is used for image analysis algorithms, which later helped in understanding the spatial integration of soil properties, RSA traits and related gene expression in plants. Thus, emerged as a system that has great utility in presenting environmental stimuli to roots in ways that evoke natural adaptive responses and in providing tools for studying the multi-dimensional nature of such processes. Some other plants on which this technique was used to study the root system architecture are Tomato (*Lycopersicum esculentum*) and grasses, Rellán-Álvarez et al. (2015).

X-ray computed tomography

Minimally invasive structural imaging by X-ray computed tomography (CT) method enables researchers to reconstruct the scanned objects in a three-dimensional (3-D) fashion (Fig. 1 g). Initially used as a medical diagnostic tool, this technique was first used as a medical diagnostic tool in 1971, but since then has been utilized in a wide range of scientific fields, such as natural, material and earth sciences (Stuppy et al. 2003; Cnudde et al. 2006; Teramoto et al. 2020; Shao et al. 2021). This method is widely used to visualize the plant root system growing in the natural environment. For this remarkable work, the pioneer of this technique G. N. Hounsfield was awarded a Nobel prize in 1979. The non destructive and non-invasive nature of this technique has made it a competent tool for soil profile analysis. The use of X-ray CT in plant science and soil sciences was started by Crestana and Vaz (1998). Hainsworth and Aylmore (1983) used computer-assisted tomography to determine the spatial distribution of water content in soil and also studied its role in the visualization of the root system. The technique is explored for root phenes identification under different substrates, such as sandy and clayey soils. For instance, X-ray CT is used to collect root-network images of chestnut (*Aesculus hippocastanum*) and maple (*Acer pseudoplatanus*) trees growing in sandy and clayey soils. In this study, large roots permitted the use of a global threshold approach, where seeds were germinated and allowed to grow until 2–3 weeks' time on germination sheets placed in containers containing different abiotic stressors. Interestingly, Pierret et al. (1999) were amongst the first to identify one of the major imaging challenges concerned with root segmentation. Later, Kaestner et al. (2006) endeavored to provide the solution by applying a non-linear diffusion filter to smoothen the images followed by conveying a threshold value extracted from Rosin's (2001) algorithm, and concluded that a phenotyping dilation operation could be used to eradicate misclassified objects. This method could perceive primary roots and fine lateral roots as well. Lontoc-Roy et al. (2006) observed maize (*Zea mays*) roots growth in different substrates (sandy and sieved loamy sand) in both dry and wet conditions. These tactics were applied for image analysis and created preliminary documentation of root material using the whole threshold selected values. The threshold values varied with various samples, such as the CT responses between the types and conditions (e.g., moisture content) of a roots under study. The resultant threshold values could produce a model comprised of a root system enclosed by 'clouds' of categorized voxels followed by analysis, which could iteratively improve the threshold boundaries, thus outcome similar voxels related to the whole root. The final step involves skeletonizing the region identified as the root. This illustrates, how water content affects the overall density of the plant roots when examined using X-ray CT. In another study Perret et al. (2007) developed a range of methods to estimate and analyses RSA traits such as root volume, root surface area, root length and root number using high resolution X-ray CT in chickpea (*Cicer arietinum*) grown in the sand substrate. This method was able to segregate threshold values of roots, air, and water content. The similar attenuation values between the air spaces and near root objects could create a hindrance, as air spaces are close to root sectors and therefore not eliminated by using a filter. Structural organization of X-ray CT and its functioning is explained by Paya et al. (2015). Researchers have addressed a number of limitations of previous methods. An impressive study conducted by Aravena et al. (2011), utilized a synchrotron radiation computed tomography to visualize the structure of root and root hairs. Ferreira et al. (2010) applied X- ray-CT scan to potato tubers volume using ~ 0.1 mm resolution, and obtained values were vastly correlated (0.986) with the root volumes (RV) of excavated tuber samples. However, no information could be inferred about the type of substrate used for plant growth experiments. Tracy et al. (2010) and Lucas et al. (2011) provided finer inputs, their work proposed that new X-ray CT systems could envisage thin lateral roots i.e. few micrometers. X-ray CT has been used in numerous studies for invasive root observation in the soil, which includes roots interaction in *Populus tremuloides* and *Picea mariana*, root and soil interaction in tomato and wheat. However, root growth kinetics using high-throughput images by X-ray CT has not been extensively explored yet, in spite of its importance in revealing RSA plasticity in the soil. Estimate and analyses RSA traits such as root volume, root surface area, root length and root number using high resolution X-ray CT in chickpea (*Cicer arietinum*) grown in the sand substrate.

Magnetic resonance imaging (MRI)

A plant root system is highly flexible and plastic in responding to various soil environments and other gradients. So far, RSA trait analyses focus on 2D images acquisition, a non-invasive 3D root imaging could portray the real behavior of a root system in a given soil condition. These 3D RSA traits analyses could be obtained through MRI imaging with a standard protocol including spin-Echo-Multi slice (Van et al. 2016; Bagnall et al. 2020). Root image detection through MRI is affected by different soil substrates and moisture content. Results obtained from previous studies indicate that seminal root images could be detected easily in MRI; however, the dense soils create hindrance in lateral root detection. Furthermore, soil moisture content could greatly influence plant root analysis through MRI grown under different soil substrates. Moisture content above 80% and highly dense soil can significantly hamper the visualization of lateral roots in the soil and to some extent the seminal roots. Application of MRI for the RSA studies in barley has been conducted by Pflugfelder et al. (2017). For detailed experimentation and understanding refer to Pflugfelder et al. (2017) and Bagnall et al. (2020).

Field-based methods

In field-based methods, growth conditions are minimally controlled and could provide a practical relevance in RSA studies. Field-based methods impose several challenges due to variability in the physio chemical properties of the soil. Ground-based methods are damaging, effort and time exhaustive, few among these are trenching, coring, excavating, and minirhizotron (MR) used to access roots in situ. Among non-invasive techniques, minirhizotron (MRs) are widely used in field conditions for RSA analysis.

Minirhizotron

Minirhizotron (MR) technique is extensively used to explore subterranean plant root systems (Fig. 1 h-i). MR application allows direct observations of plant roots falling in the rhizosphere zone. Besides phenotyping, in situ root studies are increasingly important to understand the factors controlling agricultural yields in diverse environmental conditions. MRs has emerged as a sound tool for understanding root responses of crop systems. In general, MRs comprises mainly three parts: a transparent tube, a camera system with storage, and a computer-processing unit (Smit et al. 2000; Nakaji et al. 2008). MR installation involves, tubes hidden in the field for roots to be monitored. The positioning and imaging process is mostly manual. After the images are acquired, they need to be processed for research, which is done manually or semi-automatically using customized or commercially available software packages (ImageJ: IJ-Rhizo (Pierret et al. 2013) and SmartRoot software (Lobet et al. 2011) (Table 1).

The MRs has been used to study species of turf grass roots (Fu et al. 2007). MR allows the observation of root growth over time for the quantification of root length and measurements of RSA. The most common morphological parameter assessed is root diameter (mm to µm); though some studies have addressed root pigmentation and branching also. MRs has helped to improve our understanding of root systems, for example, in respect of root production, longevity, root interaction and distribution (Treseder et al. 2005; Vargas and Allen 2008; Ephrath and Eizenberg 2010). MRs are also used in the measurements of root depth distribution (Baumann et al. 2005; Hendricks et al. 2006; Gandullo et al. 2021) and extensively in assessing root length.

Some conflicting reports demonstrated several factors such as soil type, soil density, tube installation technique,

Table 1 Comparison of available software's used for the root system architecture analysis

Available software's for RSA analysis	Advantages	Disadvantages	References
WinRHIZO, RootReader	Traits measured Lateral root density, root angles, appearance, branching, root surface area	Work on washed roots (destructive), problems with root overlap	Arsenault et al. (1995) Clark et al. (2013)
SmartRoot software	Root diameter, insertion-angle, root orientation, root length and root distribution	Require clear washed roots (destructive), mature roots are difficult to analyze	Lobet et al. (2011)
ImageJ: IJ-Rhizo	Generates automatically root radius distributions, root morphology than the average, offered low-cost economical software packages	Similarly, clear input of the root image is required for root system studies	(Pierret et al. 2013
DynamicRoots software	The utility of this software includes automatic analysis and computation of structural organization and dynamic traits for each root in the system enabling the quantification of growth on a fine-scale	Complexity in constructing 3D-shape of plant root	Symonova et al. (2015)
Digital imaging of root traits (DIRT)	DIRT is freely available online software that permits researchers to store images of plant roots, measure root traits of both field and lab conditions, and then share data and results within a collaborative team	Require a large set of images for RSA analysis	Das et al. (2015) Iyer-Pascuzzi et al. (2010)

replicate numbers and sampling errors could largely influence MR data authenticity and its image acquisition. The consistency of MR method to predict the physiological status of plant roots i.e., dead or alive has been recently demonstrated by Rahman et al. (2020) and Gandullo et al. (2021).

Minirhizotrons for studying below-ground interactions

MRs has been used to demonstrate below-ground interactions between roots and their mycorrhizal partners and for roots and soil fauna/plant parasite interactions. Dynamics of mycorrhizal colonization in the soil cores have been studied with MRs (Mukerji et al. 2006). Fungal structures up to the single hyphae and density estimates of ectomycorrhizae, rhizomorphs and colonies of saprophytic fungi have been demonstrated with MRs (Treseder et al. 2005; Pritchard et al. 2008; Hasselquist et al. 2010). Below-ground resource competition, mediated through root–root interaction is of wide importance in plant ecosystems (Rewald and Leuschner 2009). Since MRs can estimate root biomass and distribution, they are capable to assess the degree of competition (Jose et al. 2001; Båth et al. 2008). Mostly the studies are restricted to tree–crop interactions (Campbell et al. 1994; Gillespie et al. 2000; Gandullo et al. 2020) due to difficulties in distinguishing roots of different species in situ. In addition to root competition, below-ground parasitic interactions can also be studied by MRs (Eizenberg et al. 2005). This technique is successfully used for in situ monitoring of the early stages of the root parasite establishment and interaction with plant roots (Table 2).

Applications of non-invasive techniques in root phenotyping

Cereal and herbs

The non-invasive techniques were less explored in RSA studies in cereals and plants of commercial importance. However, by using these emerging techniques, we can explore the realistic and natural growth of root systems in cereal crops. Non-invasive techniques such as X-ray CT, minirhizotron, and MRI are very popular in root studies. Recently, a high-throughput non-invasive X-ray CT time scan analysis of up to 28 days old seedling of rice was conducted successfully, this technique enabled the detection of primary root and crown roots development within a pot with a diameter up to 20 cm (Teramoto 2020). X-ray CT is also used for the in-depth understanding of rice seed development within its panicle in a non-invasive manner in a real-time manner. The observations made were not possible with traditional methods of grain development using manual removal of panicle layers. More interestingly, results obtained during X-CT of rice panicles at different stages showed a direct correlation

Table 2 Comparison of methods employed for plant root system architecture analysis

Available methods for RSA	Advantages	Disadvantages	Plants studied	References
X-ray computed tomography	Plant root length, root surface area, and volume Report image of the whole root system	Less practical, plant roots grown in small containers only	Rice Maize Wheat	Teramoto et al. (2020); Jhala and Thaker (2015). Zhao et al. (2020); Mairhofer et al. (2017). Griffiths et al. (2022); Heeraman et al. (1997) Brown et al. (1991); Metzner et al. (2015) Rogers et al. (2016)
Growth luminescence observatory root (GLO)	Plant root kinetics, direction, and physiological activity	Luminescence-based reporters to enable studies of root architecture and gene expression patterns in soil-grown, light-shielded roots	*Arabidopsis* Tomato Grasses *Brachypodium distachyon*	Rellán-Álvarez et al. (2015); Watt et al. (2009)
Minirhizotron	Root dynamics Root morphological traits Measurements on specific root surface area	lavish, labor exhaustive (construction and analyzing data), and anomalous plant root growth	Maize pepper roots	Liedgens and Richner (2001); Lu et al. (2019). Klepper and Kaspar (1994); Dannoura et al. (2008)
Magnetic resonance imaging (MRI)	Non-invasive and allow high-quality three-dimensional imaging of roots in soil Traits measured viz. total root biomass, root length, root width, root angle and root distribution	Not all substrates, however, are suitable for MRI. Soil moisture content can cause the hindrance in root imaging	Maize Barley Rice Wheat	Van Dusschoten et al. (2016); Schulz et al. (2013). Liao et al. (2015); Van et al. (2016) ; Liu et al. (2014); Pflugfelder et al. (2022); Pflugfelder et al. (2017); Pohlmeier et al. (2013); Popova et al. (2016); Yu et al. (2020); Schulz et al. (2012)
Rhizoponics	Hydroponic rhizotrons improved to grow small plants RSA and shoot growth from seedling to vegetative stage	Nutrient control Applicable to small plants such as *Arabidopsis thaliana*	*Arabidopsis* Grapevine	Mathieu et al. (2015); Krzyzaniak et al. (2021)
Rhizoslide	Monitor plant root and shoot growth Crown root studies	Applicable to lab conditions only	Maize Durum wheat	Le et al. (2016); Boudiar et al. (2020); Le et al. (2014)
Transparent containers	Measurement of root traits such as lateral root density, presence, distribution pattern Whole root system visible, 3D, more natural architecture	Comparison between substrate i.e. soil and sand	Wheat	Neumann et al. (2009); Downie et al. (2012)

with seed dry matter, another important commercial aspect of studying non-invasive methods of RSA for rice. With the help of this wonderful technique plant breeders and farmers can decide the precise crop harvesting time for rice. Moreover, this simple and non-invasive approach has opened a window for agronomists to select root-specific traits which can be directly correlated to crop yield (Jhala and Thaker 2015). Recently, Zhao et al. (2020) constructed a 3D model of maize root system under *in-situ* conditions through X-ray CT, thus providing a geometric morphology of the maize root system. In another study, X-ray CT enabled to study RSA in wheat (*Triticum aestivum*) seedlings at different time points under high and low nitrogen concentrations. The 4D X-ray CT scanned images under soil were captured and image analysis proved that lateral root traits showed greater variation under low nitrogen conditions when compared to control conditions. This technique has helped to explore root responsive traits related to specific nutrient conditions (Griffiths 2022).

Another important non-invasive technology applied in root phenomics is MRI, though quite expensive compared to X-ray CT, this technique has better resolution in the detection of seminal and lateral roots in wheat plants. In this technique wheat seedlings were grown in natural soil; an MRI scan was conducted at different time points to note RSA variability among the different genotypes. Important traits measured through this technique were root and shoot emergence times, total root length, angle, and depth (Pflugfelder et al. 2022). Thus, MRI provided the natural and unique automated 4D images of the RSA in wheat plants and also showcased its application in other crops such as tomato, mustard, rice etc. Besides, X-ray CT and MRI, another non-invasive method is minirhizotron (MR), equipment that is portable, economical, and extensively used in root phenotyping studies under field conditions. For instance, MR is used to explore maize root growth, distribution and root density at different developmental stages from three to twelve leaf stages. Observations obtained from MR images have shown that root density has increased with depth maximally at 25 cm and reduced at greater depths (Liedgens and Richner 2001). Similarly, Lu et al. (2019) calculated non-invasively root traits i.e. root length, root surface area root volume, lateral root density, etc. in pepper root using the MR technique. From the above evidence, it may be proposed that deep learning algorithms and approaches to object recognition can be set up in order to identify monocot and dicot root systems (Yu et al. 2020).

Tree species

The RSA analysis in tree species is least explored because of the complex root system and its wider distribution in soil. However, some non-invasive RSA studies on root phenotyping of tree species have been conducted successfully using a newly developed electrical current source approach in the Citrus plant. In this approach a drift of the electric current in the root system is related to the pathways of water and solutes, thus providing information on the root architecture and its functioning (Peruzzo et al. 2020). The PhenoRoots has been used to assess the variability of the RSA in cotton and has shown variation in root attributes at different soil profiles and soil depths (Martins et al. 2019). Further, the MR technique has been extended to tree RSA through improved computational algorithms. More recently, a modified version of the MR technique has been patented for non-invasive root phenotyping in small-sized trees (Moore et al. 2022). This latest technique has better resolution in capturing the root attributes in a real-time manner (Moore et al. 2022). In tree species applications of X-CT and MR are limited and require modifications in terms of portability and sensor development then only these techniques could be applied for higher plant root studies.

Conclusions and future explorations

The RSA trait identification plays a pivotal role in crop improvement programs. In-depth analysis of RSA is an urgent need to use the latest molecular technologies, to collaborate the knowledge of root studies and breeding programs. Some interesting RSA traits viz. water use efficiency and nitrogen use efficiency are highly desired for the generation of super crops. Aerial and below-ground plant phenotyping in combination will allow the selection of specific genotype and practices on farms to enhance productivity gain. However, this requires a paradigm shift in the development of new approaches, timelines and intensity of research work programs related to RSA phenotyping. The present article highlights the advantages and discrepancies of available systems developed for plant root phenotyping. For instance, hydroponics does not permit precise and practical relevant root quantification, despite 2D-rhizotron being particularly suitable for root traits quantification, root-shoot physiological relations, and root system responses to local soil conditions. The rhizoslide method allows studying the root growth of crown roots and seminal roots independently under heterogeneous environmental conditions. The GLO Roots enable studies of root architecture and gene expression patterns in soil-grown and light-shielded roots. Further 3D imaging by X-ray CT and MRI techniques provides the most applicable and practical relevance for quantifying RSA traits. Non-invasive study of RSA (X-ray CT, MRI, Minirhizotron) is one of the greatest challenges in tracing the important contributing root traits under different environmental stimuli. This review has presented the latest developments in the field of RSA studies. A comparison of different methods available to date will also help to understand the shortcomings of the methods/experiments in practice (Table 2). From every point of concern, X- ray computed tomography may be used to capture high-throughput images, but economical design, flexibility, and portability remain challenging task for its wider application. Further, there is a need to explore sensor-based techniques viz. thermal imaging sensor and ultrasound in exploring the interactions of different environmental stimuli with RSA.

Data resources utilized in the review process

An in-depth literature survey was carried to understand the recent methods of RSA studies. A comparison of X-Ray CT, GLO-Root, minirhizotron, MRI, rhizoponics, rhizoslide, and transparent containers is shown in Tables 2. An extensive search has been carried out in peer-reviewed publications, field guides, books, conference proceedings, project reports, and other information available on the internet. Numerous software packages (ImageJ: IJ-Rhizo (Pierret et al. 2013) and SmartRoot software (Lobet et al. 2011) were analyzed in extracting quantitative root traits from captured images. Several online websites with dedicated software's viz.https://github.com/st707311g/RSAtrace3D, https://www.quantitativeplant.org/software/root-vis, https://www.quantitative-plant.org/software/giaroots are available for analyzing the RSA of plants of commercial importance.

Acknowledgements Authors would like to thank Department of Botany, University of Jammu for extending laboratory facilities and technical support to SP. Further thanks to CSIR for providing research fellowship to MU.

Declarations

Conflict of interest The authors declare no conflict of interest relating to financial interests or personal relationship that could have appeared to influence the work reported in this paper.

References

Adu MO, Chatot A, Wiesel L et al (2014) A scanner system for high-resolution quantification of variation in root growth dynamics of *Brassica rapa* genotypes. J Exp Bot 65(8):2039–2048

Alemu A, Feyissa T, Maccaferri M et al (2021) Genome-wide association analysis unveils novel QTLs for seminal root system architecture traits in *Ethiopian durum* wheat. BMC Genom 22(1):1–16

Aravena JE, Berli M, Ghezzehei TA, Tyler SW (2011) Effects of root-induced compaction on rhizosphere hydraulic Properties-X-ray microtomography imaging and numerical simulations. Environ Sci Technol 45(2):425–431

Arsenault JL, Poulcur S, Messier C, Guay R (1995) WinRHIZO™, a root-measuring system with a unique overlap correction method. HortScience 30:906

Bagnall GC, Koonjoo N, Altobelli SA et al (2020) Low-field magnetic resonance imaging of roots in intact clayey and silty soils. Geoderma 370:114356

Bao Y, Wang C, Jiang C et al (2014) The tumor necrosis factor receptor-associated factor (TRAF)-like family protein SEVEN IN ABSENTIA 2 (SINA2) promotes drought tolerance in an ABA-dependent manner in *Arabidopsis*. New Phytol 202(1):174–187

Baumann DL, Workmaster BA, Kosola KR (2005) Ben Lear' and Stevens' Cranberry root and shoot growth response to soil water potential. HortScience 40(3):795–798

Boudiar R, González J, Mekhlouf A et al (2020) Durum wheat seminal root traits within modern and landrace germplasm in Algeria. Agronomy 10(5):713

Brown DP, Pratum TK, Bledsoe C, Ford DE, Cothern JS, Perry D (1991) Noninvasive studies of conifer roots: Nuclear magnetic resonance (NMR) imaging of Douglas-fir seedlings. Can J For Res 21:1559–1566

Båth B, Kristensen HL, Thorup-Kristensen K (2008) Root pruning reduces root competition and increases crop growth in a living mulch cropping system. J Plant Interact 3(3):211–221

Campbell CD, Mackie-Dawson LA, Reid EJ et al (1994) Manual recording of minirhizotron data and its application to study the effect of herbicide and nitrogen fertilizer on tree and pasture root growth in a silvopastoral system. Agrofor Syst 26(2):75–87

Chen BC, Legant WR, Wang K et al (2014) Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science 346:6208

Clark RT, Famoso AN, Zhao K et al (2013) High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant Cell Environ 36(2):454–466

Cnudde V, Masschaele B, Dierick M et al (2006) Recent progress in X-ray CT as a geosciences tool. Appl Geochem 21(5):826–832

Conn SJ, Hocking B, Dayod M et al (2013) Protocol: optimizing hydroponic growth systems for nutritional and physiological analysis of Arabidopsis thaliana and other plants. Plant Methods 9(1):1–1

Crestana S, Vaz CMP (1998) Non-invasive instrumentation opportunities for characterizing soil porous systems. Soil Tillage Res 47(1–2):19–26

Dannoura M, Kominami Y, Oguma H, Kanazawa Y (2008) The development of an optical scanner method for observation of plant root dynamics. Plant Root 2:14–18

Das A, Schneider H, Burridge J, Ascanio AKM, Wojciechowski T, Topp CN, Bucksch A (2015) Digital imaging of root traits (DIRT): a high-throughput computing and collaboration platform for field-based root phenomics. Plant Methods 11(1):1–12

Downie H, Holden N, Otten W, Spiers AJ, Valentine TA, Dupuy LX (2012) Transparent soil for imaging the rhizosphere. PLoS ONE 7:e44276

Eizenberg H, Shtienberg D, Silberbush M et al (2005) A new method for in-situ monitoring of the underground development of *Orobanche cumana* in sunflower (*Helianthus annuus*) with a mini-rhizotron. Ann Bot 96(6):1137–1140

Ephrath JE, Eizenberg H (2010) Quantification of the dynamics of *Orobanche cumana* and *Phelipanche aegyptiaca* parasitism in confectionery sunflower. Weed Res 50(2):140–152

Ferreira SJ, Senning M, Sonnewald S et al (2010) Comparative transcriptome analysis coupled to X-ray CT reveals sucrose supply and growth velocity as major determinants of potato tuber starch biosynthesis. BMC Genom 11(1):1–17

Fu J, Fry J, Huang B (2007) Tall fescue rooting as affected by deficit irrigation. HortScience 42(3):688–691

Gandullo J, Ahmad S, Darwish E et al (2021) Phenotyping tomato root developmental plasticity in response to salinity in soil rhizotrons. Plant Phenom, 2021

Gangopadhyay M, Das AK, Sahu R et al (2021) Evaluation of growth response for mass production and accumulation of 2-hydroxy-4-methoxybenzaldehyde in endangered *Hemidesmus indicus* by an aeroponic system. Ind Crops Prod 172:114072

Griffiths M, Mellor N, Sturrock CJ et al (2022) X-ray CT reveals 4D root system development and lateral root responses to nitrate in soil. The Plant Phenome J, 5(1), e20036

Hainsworth JM, Aylmore LAG (1983) The use of computer assisted tomography to determine spatial distribution of soil water content. Soil Res 21(4):435–443

Hakla HR, Sharma S, Urfan M et al (2021) Gibberellins target shoot-root growth, morpho-physiological and molecular pathways to induce cadmium tolerance in *Vigna radiata* L. Agronomy 11(5):896. https://doi.org/10.3390/agronomy11050896

Hasselquist NJ, Vargas R, Allen MF (2010) Using soil sensing technology to examine interactions and controls between ectomycorrhizal growth and environmental factors on soil CO 2 dynamics. Plant Soil 331(1):17–29

Heeraman DA, Hopmans JW, Clausnitzer V (1997) Three-dimensional imaging of plant roots in situ with X-ray computed tomography. Plant Soil 189:167–179

Hendricks JJ, Hendrick RL, Wilson CA et al (2006) Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94(1):40–57

Hochholdinger F, Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. Curr Opin Plant Biol 12(2):172–177

Iyer-Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Benfey PN (2010) Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Physiol 152(3):1148–1157

Jhala VM, Thaker VS (2015) X-ray computed tomography to study rice (*Oryza sativa* L.) panicle development. J Exp Bot 66(21):6819–6825

Jose S, Gillespie AR, Seifert JR et al (2001) Comparison of minirhizotron and soil core methods for quantifying root biomass in a temperate alley cropping system. Agrofor Syst 52(2):161–168

Kaestner A, Schneebeli M, Graf F (2006) Visualizing three-dimensional root networks using computed tomography. Geoderma 136(1–2):459–469

Kano M, Inukai Y, Kitano H et al (2011) Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. Plant Soil 342(1):117–128

Kell DB (2011) Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. Ann Bot 108(3):407–418

Klepper B, Kaspar TC (1994) Rhizotrons: their development and use in agricultural research. Agron. J, 86, 745–753. Plants 2015, 4390

Krzyzaniak Y, Cointault F, Loupiac C et al (2021) In situ phenotyping of grapevine root system architecture by 2D or 3D imaging: advantages and limits of three cultivation methods. Front Plant Sci,1278

LaRue TS (2020) Uncovering natural variation in root system architecture dynamics. Stanford University, Stanford

Leitner D, Felderer B, Vontobel P et al (2014) Recovering root system traits using image analysis exemplified by two-dimensional neutron radiography images of lupine. Plant Physiol 164(1):24–35

Liao R, Bai Y, Liang H et al (2015) Root growth of maize as studied with minirhizotrons and monolith methods. Arch Agron Soil Sci 61(10):1343–1356

Liedgens M, Richner W (2001) Minirhizotron observations of the spatial distribution of the maize root system. Agron J 93(5):1097–1104

Lobet G, Pagès L, Draye X (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol 157(1):29–39

Lontoc-Roy M, Dutilleul P, Prasher SO et al (2006) Advances in the acquisition and analysis of CT scan data to isolate a crop root system from the soil medium and quantify root system complexity in 3-D space. Geoderma 137(1–2):231–241

Lu W, Wang X, Wang F (2019) Adaptive minirhizotron for pepper roots observation and its installation based on root system architecture traits. Plant Methods 15(1):1–14

Lucas M, Swarup R, Paponov IA et al (2011) Short-Root regulates primary, lateral, and adventitious root development in *Arabidopsis*. Plant Physiol 155(1):384–398

Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109(1):7

Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. Philos Trans R Soc B: Biol Sci 367(1595):1598–1604

Mahanta D, Rai RK, Mishra SD et al (2014) Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. Field Crops Res 166:1–9

Mairhofer S, Pridmore T, Johnson J et al (2017) X-ray computed tomography of crop plant root systems grown in soil. Curr Protoc Plant Biol 2(4):270–286

Le Marié C, Kirchgessner N, Marschall D et al (2014) Rhizoslides: paper-based growth system for non-destructive, high throughput phenotyping of root development by means of image analysis. Plant Methods 10(1):1–16

Martins S, Brito M, Gonçalves GGD, Tripode WDC, Lartaud BMD et al (2019) PhenoRoots: an inexpensive non-invasive phenotyping system to assess the variability of the root system architecture. *Scientia Agricola*, *77*

Mathieu L, Lobet G, Tocquin P et al (2015) "Rhizoponics": a novel hydroponic rhizotron for root system analyses on mature *Arabidopsis thaliana* plants. Plant Methods, 11(1), 1–8

Metzner R, Eggert A, van Dusschoten D et al (2015) Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: potential and challenges for root trait quantification. Plant Methods 11(1):1–1

Moore M, Aguilar J, Johnson L (2022) U.S. patent Application No. 17/421,369

Mukerji KG, Manoharachary C, Singh J (eds) (2006) Microbial activity in the rhizosphere, vol 7. Springer, Berlin

Nakaji T, Noguchi K, Oguma H (2008) Classification of rhizosphere components using visible–near infrared spectral images. Plant Soil 310(1):245–261

Narayanan S, Mohan A, Gill KS et al (2014) Variability of root traits in spring wheat germplasm. PLoS ONE, 9(6), e100317

Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere—the plant science toolbox. Plant Soil 321:431–456

Pal S, Mushtak K, Christian D et al (2017) Transdetect identifies a new regulatory module controlling phosphate accumulation. Plant Physiol,175 916–926

Paya AM, Silverberg JL, Padgett J et al (2015) X-ray computed tomography uncovers root–root interactions: quantifying spatial relationships between interacting root systems in three dimensions. Front Plant Sci 6:274

Perelman A, Jorda H, Vanderborght J et al (2020) Tracing root-felt sodium concentrations under different transpiration rates and salinity levels. Plant Soil 447(1):55–71

Perret JS, Al-Belushi ME, Deadman M (2007) Non-destructive visualization and quantification of roots using computed tomography. Soil Biol Biochem 39(2):391–399

Peruzzo L, Chou C, Wu Y, Schmutz M, Mary B, Wagner FM, Hubbard S (2020) Imaging of plant current pathways for non-invasive root phenotyping using a newly developed electrical current source density approach. Plant Soil 450(1):567–584

Pflugfelder D, Kochs J, Koller R et al (2022) The root system architecture of wheat establishing in soil is associated with varying elongation rates of seminal roots: quantification using 4D magnetic resonance imaging. J Exp Bot 73(7):2050–2060

Pflugfelder D, Metzner R, van Dusschoten D et al (2017) Non-invasive imaging of plant roots in different soils using magnetic resonance imaging (MRI). Plant Methods 13(1):1–9

Pierret A, Capowiez Y, Moran CJ et al (1999) X-ray computed tomography to quantify tree rooting spatial distributions. Geoderma 90(3–4):307–326

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(1):531–539

Pohlmeier A, Javaux M, Vereecken H, Haber-Pohlmeier S, Anderson SH, Hopmans JW (2013) Magnetic resonance imaging techniques for visualization of root growth and root water uptake processes. In: Anderson SH, Hopmans JW (eds) SSSA special publication. Soil Science Society of America Inc, Madison, USA

Popova L, van Dusschoten D, Nagel KA, Fiorani F, Mazzolai B (2016) Plant root tortuosity: an indicator of root path formation in soil with diferent composition and density. Ann Bot 118(4):685–698

Pritchard SG, Strand AE, McCormack ML et al (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO2-enrichment: a six-year-minirhizotron study. Glob Chang Biol 14(3):588–602

Rahman G, Sohag H, Chowdhury R et al (2020) SoilCam: a fully automated minirhizotron using multispectral imaging for root activity monitoring. Sensors 20(3):787

Rellán-Álvarez R, Lobet G, Lindner H et al (2015) GLO-Roots: an imaging platform enabling multidimensional characterization of soil-grown root systems.Elife, 4, e07597

Rewald B, Leuschner C (2009) Does root competition asymmetry increase with water availability? Plant Ecol Divers 2(3):255–264

Richard CA, Hickey LT, Fletcher S et al (2015) High-throughput phenotyping of seminal root traits in wheat. Plant Methods 11:13. https://doi.org/10.1186/s13007-015-0055-9

Rogers ED, Monaenkova D, Mijar M et al (2016) X-ray computed tomography reveals the response of root system architecture to soil texture. Plant Physiol 171(3):2028–2040)

Rosin PL (2001) Unimodal thresholding. Pattern Recogn 34(11):2083–2096

Schulz H, Postma JA, Dusschoten DV (2013) Plant root system analysis from MRI images. Computer vision, imaging and computer graphics. Theory and application. Springer, Berlin, pp 411–425

Schulz H, Postma JA, Van Dusschoten D(2012) 3D reconstruction of plant roots from MRI images. In VISAPP (**2**) (pp. 24–33)

Shao MR, Jiang N, Li M et al (2021) Complementary phenotyping of maize root architecture by root pulling force and X-ray computed tomography. bioRxiv

Sharma A, Madhu B, Mohammad U et al (2021) Nickel excess affects phenology and reproductive attributes of *Asterella wallichiana* and *Plagiochasma appendiculatum* growing in natural habitats. Sci Rep 11:1–14

Smit AL, George E, Groenwold J (2000) Root observations and measurements at (transparent) interfaces with soil. Root methods. Springer, Berlin, pp 235–271

Stuppy WH, Maisano JA, Colbert MW et al (2003) Three-dimensional analysis of plant structure using high-resolution X-ray computed tomography. Trends Plant Sci 8(1):2–6

Symonova O, Topp CN, Edelsbrunner H (2015) DynamicRoots: a software platform for the reconstruction and analysis of growing plant roots. PLoS One, 10(6), e0127657

Teramoto S, Takayasu S, Kitomi Y et al (2020) High-throughput three-dimensional visualization of root system architecture of rice using X-ray computed tomography. Plant Methods 16:1–14

Tiziano B, Giovanni B, Alberto P (2021) Maize plant (*Zea mays*) uptake of organophosphorus and novel brominated flame retardants from hydroponic cultures. Chemosphere,132456

Tracy SR, Roberts JA, Black CR et al (2010) The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. J Exp Bot 61(2):311–313

Treseder KK, Allen MF, Ruess RW et al (2005) Lifespans of fungal rhizomorphs under nitrogen fertilization in a pinyon-juniper woodland. Plant Soil 270(1):249–255

Uga Y, Sugimoto K, Ogawa S et al (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45(9):1097–1102

Urfan M, Haroon RH, Shubham S et al (2021) Root specific plasticity induced by paclobutrazol confers improved deficit irrigation tolerance and agronomic performance in maize. *bioRxiv* (2021): 2020-05.https://doi.org/10.1101/2020.05.12.087940

Van Dusschoten D, Metzner R, Kochs J et al (2016) Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. Plant Physiol 170(3):1176–1188

Vargas R, Allen MF (2008) Environmental controls and the influence of vegetation type, fine roots and rhizomorphs on diel and seasonal variation in soil respiration. New Phytol 179(2):460–471

Yu G, Zare A, Sheng H et al (2020) Root identification in minirhizotron imagery with multiple instance learning. Mach Vis Appl 31(6):1–1

Zhao X, Xing L, Shen S et al (2020) Non-destructive 3D geometric modeling of maize root-stubble in-situ via X-ray computed tomography. Int J Agric Biol Eng 13(3):174–179)