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Finding the best method for visualizing the root system architecture

(Deja-Muylle *et al*, 2021)

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Summary

Plants and trees are present in all sizes, colors and shapes, the one more remarkable than the other. The shoots of plants are relatively easy to monitor, since they are the above the ground portion of the plants. A lot of things can be interpreted about the plant only by looking at the shoot. However, this is only half of the plant while there is a whole root system under the ground. Where most of the nutrients are gathered, water is taken up and potentially symbiotic associations are taking place. Therefore, the root system is also an important aspect of interpreting the whole plant and a lot of factors are contributing to the shape of the root system architecture. Although, visualizing the root system may not be as simple as monitoring the shoot. Since, it is integrated into the soil. Researchers have used several methods to investigate and visualize the root system architecture of a plant. However, which method is the best?

A “golden ratio” was set based on a few parameters which are important for the effectiveness and reproducibility of a technique. Based on these parameters could be concluded which technique was better than the other techniques.

It was not possible to appoint a method to be the best method. Since, some techniques would give a better interpretation based on which source/detector they use (e.g. electromagnetic waves or an electric current). Therefore are simply better suited for measuring certain parameters of the RSA of a plant. While other techniques were better in measuring another parameter. A promising technique for visualizing the RSA is EIT, but it still needs improvement. Using a technique mostly depends on the trade-offs a person (researcher) makes depending on their own preferences. Are you willing to sacrifice in resolution, while a technique is cheaper and quicker compared to the other technique? Or do you need the best resolution no matter the cost and duration of the technique?

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Introduction

Plants and trees are present in all sizes and colors, the one more remarkable than the other. The shoots of plants are relatively easy to monitor, since they are the above the ground portion of the plants. A lot of things can be interpreted about the plant only by looking at the shoot. However, this is only half of the plant while there is a whole root system under the ground. Where most of the nutrients are gathered, water is taken up and potentially symbiotic associations are taking place. Therefore, the root system is also an important aspect of interpreting the whole plant and a lot of factors are contributing to the shape of the root system architecture (RSA). Although, visualizing the root system may not be as simple as monitoring the shoot. Since, it is integrated into the soil. Researchers have used several methods to investigate and visualize the root system architecture of a plant, the one method being better than the other.

Several factors are contributing to changes in shape of the root system architecture and these are elaborated more thoroughly in the following parts. This is to indicate the complexity of the root system of a plant.

The first factor is nutrients, nutrients are an important factor for root growth and root development. The distribution of nutrients in not homogenously spread across the soil (Kuijken *et al*, 2015). Plant will therefore adjust the growth of their root system to the nutrient availability. Moreover, activating developmental programs that lead to changes in the root system architecture (López-Bucio *et al*, 2003). The three most important nutrients are: nitrate, phosphate and sulfate.

Nitrogen is one of the most abundant elements on earth and yet limiting for plant growth. Nitrate, the most common form of nitrogen, is highly soluble and can therefore easily be washed away or taken up by bacteria in the soil (López-Bucio *et al*, 2003). A high nitrate concentration in the soil would result in reduced lateral root elongation. In contrast, when there is an overall low nitrate concentration but locally a high nitrate concentration. Then, the plant will induce the lateral root elongation in that spot (López-Bucio *et al*, 2003).
Phosphorus is also a limiting element for plant growth. The anionic form, phosphate, is very poorly soluble due to the high affinity to cations (Ca^{2+}, Mg^{2+} and Al^{3+}) (López-Bucio *et al*, 2003). Phosphorus availability is an important factor for root hair elongation and density. If phosphorus is limitedly available then there will be a significant increase in lateral root hair length and the root hair density could increase up to five-fold the normal density (López-Bucio *et al*, 2003). While the growth of the primary root is inhibited (Péret *et al*, 2014).
The third nutrient is sulfate. When sulfate is limited, plants will develop a more branched root system. Lateral roots are then formed close to the root tip and at an increased density. When plants are supplied with sulfate the primary roots will mostly elongate. Lateral roots will elongate at a further distance from the root tip itself (López-Bucio *et al*, 2003).

Another factor is the environment. The environment is an important contributor to the shape of a plant his root system. Like explained earlier, there are several biotic factors that affect the development of the RSA. However, there are also abiotic factors that are even as important. Examples are temperature, salinity, wind and drought (Piñeros *et al*, 2016) and (Yasrab *et al*, 2019). The key components for creating a 3-D visualization of the root system architecture, root growth, branching, surface area and angle, are continuously adjusted to the signals from biotic and abiotic factors of the environment (Morris *et al*, 2017). This is to optimize resource capture (Morris *et al*, 2017).
A way of controlling the root environment is by excreting exudates. About 20-50% of the total produced assimilates is translocated to the roots. In the roots, about 10-18% of the carbon is released into the soil by root exudation (Kuijken *et al*, 2015). Under field conditions play these exudates a significant role in a plethora of purposes (Kuijken *et al*, 2015). Like for instance the nutrient acquisition of a plant. However, under highly controlled conditions can these exudates be seen as a waste (Kuijken *et al*, 2015). Nonetheless, exudate excretion is an important factor

in shaping the RSA. Being able to identify these different exudates and being able to quantify targeted sites has potential for breeding purposes (creating favorable exudation profiles).
Constructing the root system of a plant via invasive or noninvasive sampling techniques is a way of phenotyping the root system. Differences in phenotype can be caused by a low reproducibility. This low reproducibility is due to high environmental variation for one specific genotype, i.e. macro-environmental variation. Differences between repeated samples of an individual genotype within the same phenotyping platform is called micro-environmental variation (Kuijken *et al*, 2015). When the amount of micro-environmental variation is high then the heritability of that genotype be very low. Even though, macro- and micro-environmental factors can interact with several genetic factors. Thereby, undermining heritability of traits and obscuring the relation between the traits measured on a phenotyping platform (Kuijken *et al*, 2015).
While there are differences within a group of species, there are also differences between species. In *Arabidopsis thaliana*, a dicotyledonous plant, the embryo-derived primary root remains active throughout the plant his life cycle. While in monocotyledonous plants, like *Triticum aestivum*, the seedling forms seminal roots to obtain nutrients and provides anchorage (Morris *et al*, 2017).
Understanding how roots develop in different environments is only a part of the whole picture. A lot of research is done on finding which genes are important in RSA development. A new set of studies, called genome-wide association studies (GWAS), associates information of the genotype with the phenotype (Deja-Muylle *et al*, 2021). The information is gathered from a large population which is known to have a natural variability of traits. Due to next generation sequencing is it possible to sequence a large amount of plant species. GWAS uses statistical association of a trait and a single nucleotide polymorphism (SNP) and tests if these are phenotypically different. The big advantage over quantitative trait loci (QTLs) is that GWAS has a much wider variability, because it also makes use of different ecotypes (Deja-Muylle *et al*, 2021).

There are different ways to map the RSA of a certain plant. The first way is by invasive sampling. Invasive sampling makes use of a probe that has been pushed through the ground right where the shoot ends and where the root system begins. Another way of invasive sampling is shovelomics, in shovelomics the root system is extracted using a shovel to investigate the root system (Wasson *et al*, 2020). The consequence of invasive sampling is that the RSA of a plant can be observed only once, due to the invasive approach.
Another way of interpreting the RSA is by, minimally invasive sampling. In this case a tube is inserted into the ground under a certain angle, where it crosses the roots of a plant. An image sensor is then used to visualize the root system of a plant (Wasson *et al*, 2020). The advantage compared to invasive sampling is that the root system can be visualized multiple times without it being severely damaged by the sampling method.

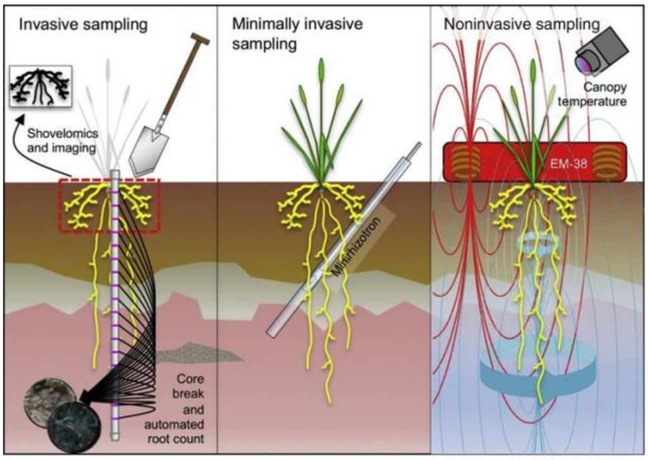

Figure 1. Different types of sampling methods. (Wasson *et al*, 2020)

The last sampling method is noninvasive sampling. Noninvasive sampling uses canopy temperature and spectral emission to visualize the RSA. Different detectors or cameras are placed at the surface (depending on the exact method). These emit electromagnetic waves and via the usage of software a 3-D visualization of the RSA can be produced. One of the major advantages is that the RSA is not damaged in any way by the sampling equipment (Wasson *et al*, 2020). Moreover, a continuous scan could be performed to investigate fluxes, of for example nutrients, within the roots.

Based on the information mentioned above can be concluded that there are a lot of factors that play a role in shaping the RSA. Moreover, there are also a lot of techniques used for visualizing the root system. However, which technique is best suited for creating a 3-D model of the RSA? Being able to answer this question is one of the main goals of this paper. X-ray CT and MRI are the most applied techniques. However, EIT could be a promising alternative if being improved over the years.

For more elaborate information about the RSA and more information about some of the mentioned techniques, (Morris *et al*, 2017) and (Kuijken *et al*, 2015) are great papers that provide you the additional information you need.

Discussion

For being able to define a “best” method, the method needs to meet certain criteria. In this literature research we used the following parameters as criteria: duration, cost, resolution, the type of sampling and being able to use software for analyzing data. These are important for visualizing the effectiveness and reproducibility of a technique. Based on these parameters could be concluded which technique was better than the other techniques. A perfect technique ultimately should take a short time from analyzing to creating a visualization of the RSA. Furthermore, should it be inexpensive and still give a high resolution while not inhibiting or affecting the RSA. In the remainder of the discussion will these parameters be discussed and placed into perspective.

As mentioned earlier can the techniques for visualizing the RSA be divided in three categories. One of them is the noninvasive techniques. A common used technique is the rhizotron. A rhizotron is a transparent column filled with soil. It often contains removable observation windows (Atkinson *et al*, 2019). In this way can a 2-D visualization of the plants RSA be created. Another important advantage of a rhizotron is that several measurements can be performed on the same roots at frequent time intervals (Nagel *et al*, 2012). The rhizotron is an example of a quick, low cost and easy to use method. In here, plants can grow inside a controlled environment while you can still inspect the RSA, through a glass pane. The major flaw of this technique is that it forces the plant to form a RSA that is more or less in 2-D, since the “disks” are very slim. Which is not a natural representation for the RSA.

In natural situations, plants will extend their roots in any direction that may favor them. Thus, creating a 3-D visualization of the RSA will be more representative and provide additional information about the growth of the RSA. When several plants are sown close together the roots will most likely mix together. This would affect the plants and could mean that plants have to adapt their RSA to still efficiently obtain nutrients and water. In agriculture most plants grow very close together, therefore understanding the growth of root systems could be a very important aspect for several purposes in optimizing agriculture. Being able to observe the growth of the RSA of a few plants in a rhizotron is already a big step. However, observing this through a noninvasive method that creates a 3-D visualization of the RSA would significantly help understanding this growth. Therefore, finding a technique that does not affect the plants, is able to continuously monitor the RSA and visualize this in 3-D could be very rewarding.

One of such techniques is X-ray computed tomography (X-ray CT). This is an imaging technique based on attenuation of X-rays to create cross-sections which can be used to reconstruct a 3-D model (Atkinson *et al*, 2019).
Another technique is magnetic resonance imaging (MRI). MRI is an imaging technique based on the absorption and re-emission of electromagnetic radiation from nuclei (in most cases hydrogen nuclei in water) in a magnetic field (Atkinson *et al*, 2019).
Positron emission tomography, or PET in short, is another technique used for imaging the RSA. The principle of PET is based on the detection of gamma radiation from tracer molecules (Atkinson *et al*, 2019). In most times is it used for visualizing the distribution of short half-life tracers. For instance, carbon isotopes which are used in plant metabolic processes (Atkinson *et al*, 2019). PET is often linked with techniques such as X-ray CT and MRI, due to a limited resolution (Atkinson *et al*, 2019).
Ground penetrating radar (GPR), is used for mapping the sub-surface structure by measuring reflection, refraction and scattering of pulses of high-frequency radio waves. The receiving antennae can be positioned directly in contact with the soil or even held above the surface (Atkinson *et al*, 2019).
Electromagnetic inductance (EMI), is another example of a geophysical technique, like GPR. It is used for the mapping of spatial soil electrical conductivity using sensors which are held above the soil surface (Atkinson *et al*, 2019).
Electrical impedance tomography (EIT), is an imaging technique based on an electrical response (in voltage) over an electric current. The electrical current changes due to properties

of the roots, the soil substrate, the moisture content and ionic strength. When the plant grows it will change these parameters and this change is detectable through the electrical current (Corona-Lopez *et al*, 2019).
Neutron computed tomography (NCT), is a technique mainly used for studying soil-root water dynamics. The principle is the same as X-ray CT, however neutrons are used instead of X-rays. Neutrons are in contrast to X-rays better attenuated by light materials like hydrocarbons or highly absorbing materials. This makes neutron imaging a great inspection tool for a wide range of applications (Datta & Hawari, 2020).

A problem for noninvasive sampling methods is the presence of air bubbles or ferromagnetic particles in the soil. Most noninvasive techniques rely on the emission of radiowaves, magnetism or an electric current. These bubbles or particles can decrease the resolution of such techniques, by creating more background noise. In an experimental set-up you would be able to get rid of these air bubbles, ferromagnetic particles and you could evenly mix the substrate. Nonetheless, you would still come across these problems with experiments in the field. When using rhizotrons as a sampling technique you would not come across these problems. Since, rhizotrons create a 2-D visual of the RSA and you can directly measure the roots instead of measuring through the soil.

Table 1. Different (non)invasive techniques used in research on the RSA of plants.

Technique	Duration	Cost	Resolution (µm)	Type of sampling	Software	Truthfulness
X-ray computed tomography	Very time consuming (Fang *et al*, 2019)	Expensive (Morris *et al*, 2017)	~0.5 (Tracy *et al*, 2010)	3-D Noninvasive	Yes	Fairly true
Magnetic resonance imaging	Fairly time-consuming (Stingaciu *et al*, 2013)	Expensive (Morris *et al*, 2017)	~390 (Stingaciu *et al*, 2013)	3-D Noninvasive	Yes	Fairly true
Positron emission tomography	Short time	Expensive	~1400 (Atkinson *et al*, 2019)	3-D Noninvasive	Yes	Fairly true
Ground penetrating radar	Fairly time-consuming	Fairly expensive	~2000 (Wu *et al*, 2014)	3-D Noninvasive	Yes	True
Electrical impedance tomography	Short time (Corona-Lopez *et al*, 2019)	Low cost (Corona-Lopez *et al*, 2019)	~100 (Aristovich *et al*, 2018)	3-D Noninvasive	Yes	Very true
Rhizotron	Short time	Low cost	~ 230 per pixel (Nagel)	2-D Noninvasive	No	Very true
Electromagnetic inductance	Short time (Whalley *et al*, 2017)	Fairly expensive	~2000 (Atkinson *et al*, 2019)	3-D Noninvasive	Yes	True
Neutron computed tomography	Fairly time-consuming	Fairly expensive	~110 (Mawodza *et al*, 2020)	3-D Noninvasive	Yes	True

A technique could have the best resolution, the shortest time for processing and give the best interpretations. However, when it costs an absurd amount of money to purchase it would still happily be switched for a cheaper alternative. According to table 1, the techniques that are the least expensive are: rhizotron and EIT. While X-ray CT, MRI and PET are the most expensive techniques.

The duration of a technique is often an important factor for committing to a certain technique. A technique that does not yield a high resolution in combination with taking a long time for analyzing the data, researchers would rather choose another technique with a shorter time frame. According to table 1, the techniques that cost the least amount of time are: PET, EMI and using rhizotrons.

The resolution of a technique is arguably one of the most important parameters. It gives you an indication to what extend you can distinguish different plant parts from one another. If you have a high resolution it makes it possible to examine, for instance, the root diameter, the root density and the amount of root hairs etc. (Stingaciu *et al*, 2013) and (Wu *et al*, 2014). According to table 1, the technique with the highest resolution (the lowest distance where two points are still distinguishable) is X-ray CT, followed by EIT and NCT.

Another parameter that is important for ranking common used methods is the truthfulness of these methods. How close are the results, gathered from a method, to the real situation in the soil. For a comparison of the truthfulness for the different methods, see table 1. 3-D noninvasive techniques are closer to the truth compared to ad 2-D method (rhizotron). Since, in regular conditions plants will grow their roots in three dimensions. The principle that techniques use also affects the truthfulness. Techniques that use ionizing radiation may (slightly) affect the RSA of plants (Wasson *et al*, 2020). Examples of techniques that rely on radiation are X-ray CT, MRI and PET. Furthermore, techniques such as X-ray CT and MRI plants sometimes need to be delivered to the imaging system (Corona-Lopez *et al*, 2019). This may cause complications and therefore lowers the truthfulness of these techniques.
According to (Atkinson *et al*, 2019) has EMI a rather high throughput. A high throughput results in a higher truthfulness, more data is analyzed per period of time. Therefore, better analyzed in real-time. The throughput of GPR is the same as EMI (Atkinson *et al*, 2019). While NCT has a high throughput but the method is not tested on different kind of soils (Mawodza *et al*, 2020). EIT has a high throughput (Corona-Lopez *et al*, 2019) which makes it a promising method. It is cheap, has a good resolution and makes no use of (possibly) ionizing radiation. However, it is a technique still in development and needs further improvement.

X-ray CT and MRI are two of the most used techniques for analyzing the RSA (Corona-Lopez *et al*, 2019). Both the techniques give a high resolution and are able to monitor continuously. However, the techniques are also expensive and very time-consuming. A technique that is less time consuming, costs less and has a high resolution is EIT. (Aristovich *et al*, 2018) were able to obtain a resolution of approximately 100 µm. Nonetheless, was this only the case in clinical trials and not with experiments based on root visualization. According to (Corona-Lopez *et al*, 2019) is the resolution for EIT obtained in experiments on the RSA rather low . At the current stage should it be seen as a complementary method towards X-ray CT and MRI which give a higher resolution.
However, the method should definitely not be neglected as it looks promising for the future. If the measurement equipment could be further optimized it would most certainly become a promising technique.
NCT has a comparable resolution as EIT. Nevertheless, is NCT still more time-consuming and it costs more compared to EIT or another alternative technique (e.g. a rhizotron).

For the purpose of yielding representative data, the type of sampling method is important. Being able to use a technique without disturbing the plant or having by taking the plant out of the soil (shovelomics) makes it possible to continuously monitor the RSA. This is a huge benefit in observing root growth and development. In table 1 almost all techniques are 3-D and noninvasive, except for the rhizotron. This is to showcase that the use of a 2-D method could still be a valid method to use, even though it may not be as representative.

Being able to use a technique for determining a wide pallet of parameters, being able to use it for different purposes and having a good resolution is an important aspect in choosing a certain technique. A wide applicability of a technique could for instance counteract the large cost of a technique. In this case it can be seen as an investment, because of the wide applicability. However, when it can only be used for one purpose people would consider choosing for another technique.

Some of the techniques listed in table 1 are better in visualizing the RSA, root branching points or root diameters, e.g. X-ray CT (Morris *et al*, 2017), MRI (Stingaciu *et al*, 2013), and GPR (Wu *et al*, 2014). While other techniques are better suited for determining contents such as: soil water content (EIT and EMI) or temperature tolerance (EIT). This makes declaring a method to be "the best" method very complicated.

Like mentioned earlier is not every technique equally suited for determining the same content. Therefore, are some techniques often linked together, an example of this is PET. PET is often used for visualizing the distribution of short half-life radioactive markers, e.g. carbon isotopes (Atkinson *et al*, 2019). Although, the resolution while using PET is relatively low. This low resolution is why PET is often linked with X-ray CT and MRI (Atkinson *et al*, 2019).
Combining techniques can give you a clearer view on what is going on in the RSA. MRI or X-ray CT can be used for observing the roots, root hairs and branch point etc. (Stingaciu *et al*, 2013) and (Wu *et al*, 2014). An additional technique such as EIT is better suited for inspecting temperature tolerance and physical detoriation (Corona-Lopez *et al*, 2019). Combining the findings or even creating an overlay that could continuously monitor the RSA results in a better overall perspective. Ultimately should the complementing technique be of low cost, like EIT. Otherwise, would the experimental set-up already cost a fortune without having started.

Another way of making a method more suitable is the usage of software. Most of the noninvasive methods use software to generate a visualization of the RSA. If the software is able to rapidly generate a simple continuous visual out of a big batch of data. Then makes it the technique less reliable on human work. Therefore, would it become a more precise technique since there is less space for human errors and it becomes less time consuming.
However, a down sight of this would be that you need a good computer that is able to handle such computational tasks. Not every computer would be able to work with such heavy software.

My personal approach would start with a simple 2-D technique like a rhizotron. For finding some preliminary results. If these are interesting and promising then choose a more representative technique, like a 3-D noninvasive technique. The choice would probably be between X-ray CT and MRI since these are simply most used in visualizing only the RSA. When parameters such as water distribution, temperature tolerance, carbon allocation or root phenotyping are relevant additions to earlier findings from X-ray CT or MRI then it is better to use other 3-D noninvasive techniques. Two of these techniques would be EIT or NCT, depending on the parameter of interest. Since these techniques are relatively cheap and quick to use. Such techniques are better optimized for one of the tasks listed earlier, rather than yielding the best resolution for visualizing the RSA and should therefore be seen as a more complementary technique. Moreover, EIT is a very promising technique because of the low cost, relatively high resolution and the truthful visualization. With further development would it be able to compete with more developed techniques such as, X-ray CT and MRI.

Using a technique mostly depends on the trade-offs a person (researcher) makes depending on their own preferences. Are you willing to sacrifice in resolution, while a technique is cheaper and quicker compared to the other technique? Or do you need the best resolution no matter the cost and duration of the technique?
Some techniques may give a better interpretation based on which source/detector they use (e.g. electromagnetic waves or an electric current). Therefore are simply better suited for measuring certain parameters of the RSA of a plant.

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