

Faculté des bioingénieurs

Quantification of plant root architecture: development of an integrated experiment-to-model pipeline

Auteur : Mariam Kerdoudi

Promoteur(s) : Pr. Guillaume Lobet (UCL/ELI/ELIA)

Lecteur(s) : Pr. Valentin Couvreur (UCL/ELI/ELIA),
Pr. Clément Saint Cast (INRAE)

Année académique 2023-2024

Mémoire de fin d'études présenté en vue de l'obtention du diplôme de
Bioingénieur : Master bioingénieur, sciences agronomiques

Table des matières

Table of figures

Table of tables

List of acronyms

1. INTRODUCTION.....	1
2. STATE OF THE ART	3
2.1. ROOT SYSTEM ARCHITECTURE AND ITS IMPORTANCE.....	3
2.2. TYPES AND ANATOMY OF ROOT SYSTEMS	4
2.2.1. <i>Classification of root systems</i>	4
2.2.2. <i>Detailed root classification</i>	5
2.3. CHALLENGES IN STUDYING THE ROOT SYSTEM ARCHITECTURE	6
2.4. THE NEED TO MODEL THE ROOT SYSTEM ARCHITECTURE.....	7
2.5. THE KEY TO SCIENTIFIC SUCCESS: THE VITAL ROLE OF PROTOCOLS	8
3. OBJECTIVES.....	9
4. METHODOLOGY.....	11
4.1. THE DEVELOPMENT OF THE R SCRIPT	11
4.1.1. <i>Data acquisition and their format</i>	11
4.1.2. <i>Creating functions</i>	11
4.2. DEVELOPING PROTOCOLS	11
4.3 USE OF ARTIFICIAL INTELLIGENCE TOOLS	12
5. RESULTS.....	13
5.1. RHIZOTRON PROTOCOL	13
5.1.1. <i>Introduction and definition</i>	13
5.1.2. <i>Schematic and dimensions</i>	14
5.1.3. <i>Material required</i>	15
5.1.4. <i>Choice of substrate</i>	15
5.1.5. <i>Conditions</i>	16
5.1.6 <i>Setting up the rhizotron</i>	17
5.1.7 <i>Root preparation for scanning</i>	17
5.1.8 <i>Tracking lines</i>	18
5.2. SHOVELOMICS PROTOCOL	19
5.2.1. <i>Materials required</i>	19
5.2.2. <i>Select a plant of interest</i>	19
5.2.3. <i>Digging</i>	19
5.2.4. <i>Root cleaning</i>	20
5.2.5. <i>Results</i>	20
5.3. ROOT SCANNING PROTOCOL.....	20
5.3.1. <i>Material required</i>	21
5.3.2 <i>Root system scan</i>	21
5.4. SCAN ANALYSIS	22
5.4.1. <i>SmartRoot: A semiautomated companion for dynamic root system analysis</i>	22
5.4.2. <i>RhizoVision: Root System Analysis through Full Automation</i>	28
5.5. ARCHISIMPLE MODEL: A DEEP DIVE INTO THE MODEL AND ITS PARAMETERS	32
5.5.1. <i>ArchiSimple model parameters</i>	36
5.5.2. <i>An example of application</i>	37

5.6 DATA ANALYSIS	39
5.6.1 Detailed explanation of the functions in the script.....	39
5.6.1.1 Exploring the Key Parameters in ASR Characterization	39
5.7 CHECKING THE SCRIPT EFFECTIVENESS	49
5.7.1 Sorghum and its many varieties	50
5.7.2 Wheat and spelt analysis.....	52
5.7.3 Spinach, butternut and pumpkin analysis	54
5.8 ANALYSIS OF ROOT MORPHOLOGICAL TRAITS ACROSS DIVERSE PLANT GENOTYPES	55
5.8.1 Plants and Varieties Analyzed	55
5.8.2 Minimum diameter	56
5.8.3 Maximum diameter	57
5.8.4 Inter-branch distance (IBD).....	58
5.8.5 The slope of the linear relationship between the diameter of a lateral root and that of its parent root (RDM).....	59
5.8.6 The coefficient of variation (CVDD).....	60
6. GENERAL DISCUSSION.....	61
6.1 RHIZOVISION: COULD IT POTENTIALLY REPLACE SMARTROOT?	62
7. CONCLUSION	64

Appendix

Acknowledgements

I would like to express my sincere gratitude to Guillaume Lobet, my supervisor, for his guidance and support throughout this project. Your expertise and encouragement have truly made a difference.

I am deeply thankful to the members of the PEPA group for their insightful questions and constructive feedback during our seminars. Your input has greatly enriched my work and helped me refine my ideas.

A special thanks to Thomas Dagbert for generously sharing his expertise and assisting me with the rhizotron protocol.

I am also grateful to my friends for their unwavering support and encouragement, and to my husband for his patience and understanding during this journey.

Thank you all for being part of this incredible experience and for making it memorable in so many ways.

Table of figures

Figure 1 : Root structure of Wheat, which is a monocot plant (left) and lupin, a dicot plant (right) (Australian Society of Plant Scientists 2018).....	4
Figure 2: The standard root terminology for monocotyledonous plants (on the left) and dicotyledonous plants (on the right), along with the corresponding terminology suggested by the International Society of Root Research (Freschet et al,2021).....	6
Figure 3 :Illustrated workflow for Utilizing the ArchiSimple Model	10
Figure 4: Diagram of rhizotron construction (Passot et al., 2016).....	14
Figure 5: Rizothron available from UCLouvain greenhouses.....	15
Figure 6: Rhizotrons in greenhouses (HenrichThuet,2023).	17
Figure 7: Root developed at end of rhizotron experiment. (HeinrichThuet,2023).	18
Figure 8: Root growth tracing (HeinrichThuet,2023).	18
Figure 9: Root cleaning set-up.	20
Figure 10: Scan settings (Degives, 2023).....	21
Figure 11: File Saving EPSON V850 Pro (Guide de l'utilisateur Perfection V800 Photo/V850 Pro).....	22
Figure 12: SmartRoot image.	22
Figure 13: The 4 layers containing the information. A is the source image, B, the morphology, C, Topology and finally, D, the annotations (Lobet et al., 2011).	23
Figure 14: Time-lapse image sequences of plants (Lobet et al., 2011).	25
Figure 15: The Analysis Options window.....	28
Figure 16: Root images obtained with ArchiSimple (Pagès et al., 2014).....	35
Figure 17: Two examples of simulated root systems. The first one being a primary root system (A) and the second, secondary root system (B) (Pagès et al.,2012).	37
Figure 18: Modeling the impact of a plough pan on the growth of root systems (Pagès et al.,2012).	38
Figure 19: Spinach, its relationship between the diameter of mother roots and daughter roots (Heinrich-Thuet, 2023).....	40
Figure 20: Script of diameter_outliers.	42
Figure 21: Script of get_minD.....	42
Figure 22: Script of get_maxD.	43
Figure 23: Script of get_IBDmean.	43
Figure 24: The creation of a new column.....	44
Figure 25: Script of get_RDM.	44
Figure 26: Script of get_CVDD.....	45
Figure 27: Script of get_EL.....	45
Figure 28: Combining different CSV files.	46
Figure 29: Functions displayed in the environment section.....	46
Figure 30: Minimum Diameter Calculation.	47
Figure 31: Maximum Diameter Calculation.	47
Figure 32: IBD Diameter Calculation.....	47
Figure 33: Script to obtain lateral.clean.	48
Figure 34: Script to obtain RDM.....	48
Figure 35: Script to obtain CVDD.	48
Figure 36: Script to obtain EL.....	48
Figure 37: Estimated Least Square Means and 95% Confidence Intervals for Different Varieties.	50
Figure 38: Slope of the linear regression of the diameter of the lateral roots vs. their mother root (Degives,2023).....	51
Figure 39: RDM of all six varieties (Degives,2023).....	51
Figure 40: EL value for the six varieties (Degives,2023).....	52
Figure 41: An example of data restructuring.	52
Figure 42: Merge all CSVs.....	53

<i>Figure 43: Environment containing the new dataframes.</i>	<i>54</i>
<i>Figure 44: Distribution of the finest root diameter according to the genotype or variety.</i>	<i>56</i>
<i>Figure 45: Distribution of the maximum root diameter according to the genotype or variety.....</i>	<i>57</i>
<i>Figure 46: Distribution of IBD according to the genotype or variety.</i>	<i>58</i>
<i>Figure 47: Distribution of RDM according to the genotype or variety.</i>	<i>59</i>
<i>Figure 48: Distribution of CVDD according to the genotype or variety.</i>	<i>60</i>
<i>Figure 49: Hoagland composition (ELIA protocole PEPA021).....</i>	<i>7</i>

Table of tables

<i>Table 1: Comparison of Root Architecture Models: Characteristics and Authors.....</i>	<i>8</i>
<i>Table 2: Measured traits and their explanation (Lobet et al., 2011).....</i>	<i>27</i>
<i>Table 3: List of all RhizoVision's features and their explanations (Seethepalli et al., 2021).</i>	<i>30</i>
<i>Table 4: ArchiSimple parameters and their units.</i>	<i>36</i>

List of acronyms

ANOVA Analysis of Variance

CSV Comma Separated Values

ROI Region of Interest

RSA Root System Architecture

1. Introduction

Root system architectures play a crucial role in understanding the interactions between plants and their environment. Their complex and diverse architecture has a direct influence on the growth, development and performance of plants in different ecosystems.

Unfortunately, given the opaque nature of the soils in which roots live and their fragility, manipulating them is no easy task. To understand these root architectures and their implications, computer simulation offers a valuable tool. In essence, simulation modelling complements traditional methods by providing information about root architecture that is difficult to obtain through direct observation alone. They are valuable for generating various configurations of the root system, making it easier to explore different scenarios, particularly in challenging environments.

Among these models, ArchiSimple, a model written in C++ and used for a great deal of analysis by both master's and PhD students at the UCLouvain, will be presented in this work.

However, the computer model requires the input of 24 parameters. To make it easier for all users of the model, a series of protocols for the manipulations required to obtain key measurements were drawn up, along with an R script for obtaining the values essential to the models. The aim of this thesis is therefore to improve the user experience for the researcher, to create a quasi-automatic pipeline and, ultimately, to be a complete syllabus or manual for the user.

Firstly, this work begins by setting the context of the problem, namely the importance of the architecture of the root structure and the reason why models are necessary.

Next, it is crucial to remember the importance of having protocols within the laboratory or greenhouses. For this reason, a section will be devoted to this, along with a series of protocols for all the necessary manipulations.

Then a more in-depth analysis of the ArchiSimple model will be carried out to create a reference document for people in the laboratory who want to use it. Similarly, the SmartRoot and RhizoVision software will also be reviewed.

Finally, once the manipulations have been successfully completed, the researcher will have a series of data to convert into input parameters for the ArchiSimple model. An R script has therefore been written so that the user can simply upload the data. A section will be devoted to explaining the functions contained in the script. A series of example applications will also be presented.

2. State of the art

2.1. Root system Architecture and its importance

Root system characteristics are essential for crop productivity and resistance. Researchers and crop breeders have done many studies about these characteristics in order to improve agricultural performance. The architecture of root systems is essential for providing plants with water and nutrients, anchoring them in the soil and protecting them against various stress factors, including toxic elements such as lead, cadmium, arsenic and mercury, as well as pathogens. In addition, roots are home to a diverse microbial community that can enhance plant growth, optimize resource utilization and influence signaling pathways regulating growth and development (Freschet et al., 2021).

Root system architecture includes the shape and structure of a plant's root system (Freschet et al., 2021). Shape refers to the spatial distribution of roots in the soil, while structure refers to the diversity and interrelationships of individual roots. As roots develop, their architecture changes, making the root system architecture an essential aspect of plant growth. Roots facilitate exchanges between soil and plant, transport resources and provide mechanical support. Because of their dynamic environment, roots must continually adapt to meet the changing needs of plants.

The root system architecture is described using a variety of traits that reflect both its shape and structure, along with its developmental processes. Whole plant root system architecture specifications include characteristics such as root length density and specific root length, while developmental characteristics include root branching density and root growth angle. Root growth angle is particularly important for longer roots which influence the spread of the root system, while root branching density affects local root length. These two characteristics are essential for understanding the architecture of the root system and its impact on plant growth and ecosystem functioning. Understanding root architecture is essential for improving agricultural productivity, as it can inform breeding programs to develop plants with more efficient root systems tailored to specific soil conditions (Freschet et al., 2021).

2.2.Types and anatomy of root systems

2.2.1. Classification of root systems

Root systems are generally divided into two main categories: fasciculate and tap-rooted (Figure 1). The primary distinction between these categories lies in the number of primary axes originating directly from the stem, which includes the hypocotyl, mesocotyl, and stolons. Fasciculate root systems feature numerous equally dominant roots, whereas tap-rooted systems possess a single dominant primary root (Freschet et al., 2021).

This straightforward classification is useful for practical purposes due to its simplicity and reliance on the proximal root system's morphology, which is typically the most accessible part for soil extraction. However, this binary classification is overly simplistic, necessitating additional criteria to accurately represent the vast diversity of root morphologies observed.

For instance, monocotyledonous plants exclusively exhibit fasciculate root systems. Within this group, further differentiation can be made based on the aggregation level of the primary roots attached to the shoot system. Fasciculate systems can be categorized as caespitose (dense) when roots are closely clustered or diffuse when roots are more widely spaced, as seen along a rhizome (Freschet et al., 2021).

In contrast, gymnosperms and dicotyledonous plants exhibit a wider variety of root types, often including intermediate forms. These plants typically have a dominant taproot along with several less dominant major roots. Many dicotyledonous species initially develop a prominent taproot, which may lose its dominance as the plant matures (Freschet et al., 2021).



Figure 1 : Root structure of Wheat, which is a monocot plant (left) and lupin, a dicot plant (right) (Australian Society of Plant Scientists 2018).

2.2.2. Detailed root classification

Different perspectives on root systems have led to several classifications and enriched terminology. One widely recognized classification is based on the origin and timing of root emergence, as defined by the International Society of Root Research (ISRR). This classification identifies four major root classes (Freschet et al., 2021):

- Tap Root: Develops from the primary embryonic root.
- Basal Root: Forms from the hypocotyl or mesocotyl region.
- Shoot-Borne Root: Grows from the nodes of leaves or sections between nodes.
- Lateral Root: Originates from any of the main root classes defined earlier.

In fact, as early as the embryonic stage, roots such as seminal roots are already present in the seed. The number of seminal roots varies from species to species; for example, many dicotyledons have a single seminal root (the radicle) while Poaceae species such as maize may have several. The central seminal root, often called the radicle or primary root, develops early and has a central position in the root system. When this root remains central and dominant, it is called a taproot. By comparison, other seminal roots are referred to as basal roots (Freschet et al., 2021).

Roots branch out to form lateral, secondary or branch roots, and lateral roots can form on any type of root, including other lateral roots. Many plants also develop roots directly from their shoot system, known as adventitious roots or shoot roots. These may appear at different positions on the shoots and are classified and called crown roots or nodal roots. (Figure 2)

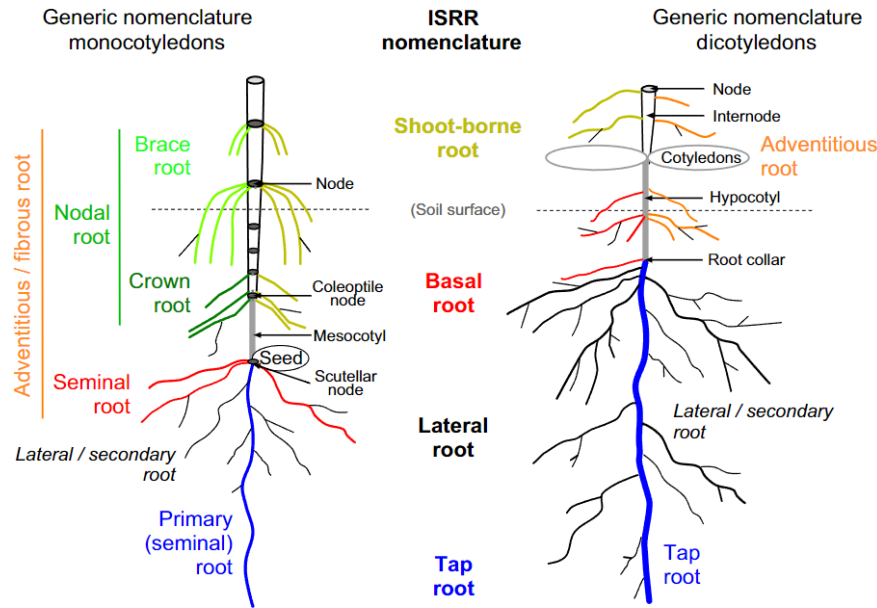


Figure 2: The standard root terminology for monocotyledonous plants (on the left) and dicotyledonous plants (on the right), along with the corresponding terminology suggested by the International Society of Root Research (Freschet et al,2021).

2.3. Challenges in studying the root system architecture

Understanding root system architecture offers significant challenges due to the complex nature of roots and the opaque soil environment. Roots display complex growth patterns within the soil matrix, making direct observation and quantification difficult without disrupting their natural arrangement or obstructing subsequent analyses.

To make matters worse, root systems are made up of a multitude of individual root axes characterized by varying degrees of development, physiology and morphology. This heterogeneity requires a nuanced understanding of the root system architecture. Much of our knowledge of root system architecture comes from theoretical models rather than empirical evidence, due to the interdependent and dynamic nature of root characteristics. Simulation models have become indispensable tools for simulating root development and analyzing the influence of various traits on root system architecture and root function. (Freschet et al., 2021).

2.4. The need to model the root system architecture

One of the main objectives shared by many modeling approaches is to improve root system representations, notably for crop models (Paez-Garcia et al., 2015). Another objective is to explore the morphology and morphogenesis of the root system independently (Pagès, 2000).

Indeed, we now understand that the root system architecture modeling facilitates comparisons between genotypes, elucidates the effects of environmental factors on root development and provides valuable information on the dynamics of carbon allocation at root system level.

Root architecture models like SimRoot (Lynch et al., 1997), RootBox (Leitner et al., 2010), RootTyp (Pagès et al., 2004), ArchiSimple (Pagès et al., 2013), OpenSimRoot (Postma et al., 2017), and CRootBox (Schnepf et al., 2018) have been developed to simulate root systems using input parameters derived from limited traits. These models are valuable for generating diverse root system architectures, enabling exploration of various configurations, especially in challenging environments (Passot, 2018).

However, not all models will be covered in this work, but it is still important to be aware of what already exists in the world of root modelling to understand the issues involved.

To provide a more visual representation of the number of existing models, their main characteristics, and their authors, a table inspired by that of Schnepf et al. (2018) has been established (Table 1).

Table 1: Comparison of Root Architecture Models: Characteristics and Authors

Model name	Authors	Type of model
ROOTMAP	Diggle (1988)	Root system
SimRoot	Lynch et al. (1997)	Root system
ArtRoot	Spek (1997)	Root system
GRAAL	Drouet and Pagès (2003)	Whole-plant structure and plant carbon flow
Root Typ	Pagès et al. (2004)	Root system
SPACSYS	Wu (2007)	Ecological model, RSA submodel
R-SWMS	Javaux et al. (2008)	Coupled soil and root system
RootBox	Leitner et al. (2010a)	Root system
PlaNet-Maize	Lobet et al. (2014)	Whole-plant structure and plant water flow
ArchiSimple	Pagès et al. (2014)	Root system
OpenSimRoot	Postma et al. (2017)	Coupled soil and root system

2.5. The key to scientific success: The vital role of protocols

We have just seen in the previous sections the importance of the root system architecture, the need to model it, existing models. The missing ingredient is the data set. To achieve this, a good deal of experience is required, and in order to meet the objective of this master thesis, which is, as a reminder, to improve the user experience when using the ArchiSimple model and therefore to improve the process of determining its parameters, a series of protocols aimed at guiding the user will also be included in this work.

Indeed, protocols, according to the Cambridge dictionary, define the rules to be followed in scientific studies or medical process. They solve the recurring problem of the lack of detailed information on methodologies, guaranteeing step-by-step instructions for experiments. This facilitates the reproducibility of experiments, the verification of results, the identification of trends and the advancement of knowledge. By specifying measurements, procedures and conditions, they ensure accurate and reliable results, reducing variability between experiments. They also include safety instructions to protect researchers and maintain a safe working environment.

3. Objectives

The main objectives of this master thesis cover a wide range of goals focused on improving the understanding and practical application of root system architecture models, particularly the ArchiSimple model.

Firstly, this master thesis aims to provide a visualisation of root system architectures and their critical role in plant growth, development and ecosystem functioning. Indeed, at this stage of the work, we understand that this is crucial for researchers and crop breeders seeking to optimise agricultural performance through informed manipulation of root characteristics.

A key objective is to improve the user experience of the ArchiSimple model. This will be achieved by developing a quasi-automatic pipeline that simplifies the process of generating input parameters. By creating a user-friendly manual and a comprehensive syllabus, this thesis will guide researchers and, in particular, students writing their master thesis in the efficient use of the ArchiSimple model, making it, then, more accessible and practical for users with different levels of expertise.

So, to arrive at this version of a syllabus, another key point is to design and document a series of detailed protocols for measuring and manipulating root systems. These protocols will provide clear, step-by-step instructions for obtaining the precise data required for the ArchiSimple model. The aim of standardising these procedures in the greenhouse is to guarantee the reproducibility and reliability of the experimental results.

The integration of IT tools into the workflow is a key area of focus. This work will integrate and explain SmartRoot and RhizoVision software for root system analysis, popularising how they work and how they obtain essential measurements for the ArchiSimple model. In addition, it aims to develop an R script to automate the conversion of experimental data into input parameters for the ArchiSimple model, demonstrating its use through practical examples.

For a student who is just getting to grips with the world of root structure analysis, and for a better understanding of what this dissertation is trying to achieve. Figure 3 has been designed to illustrate the steps involved in using the model, and thus to summarise my work in a single image.

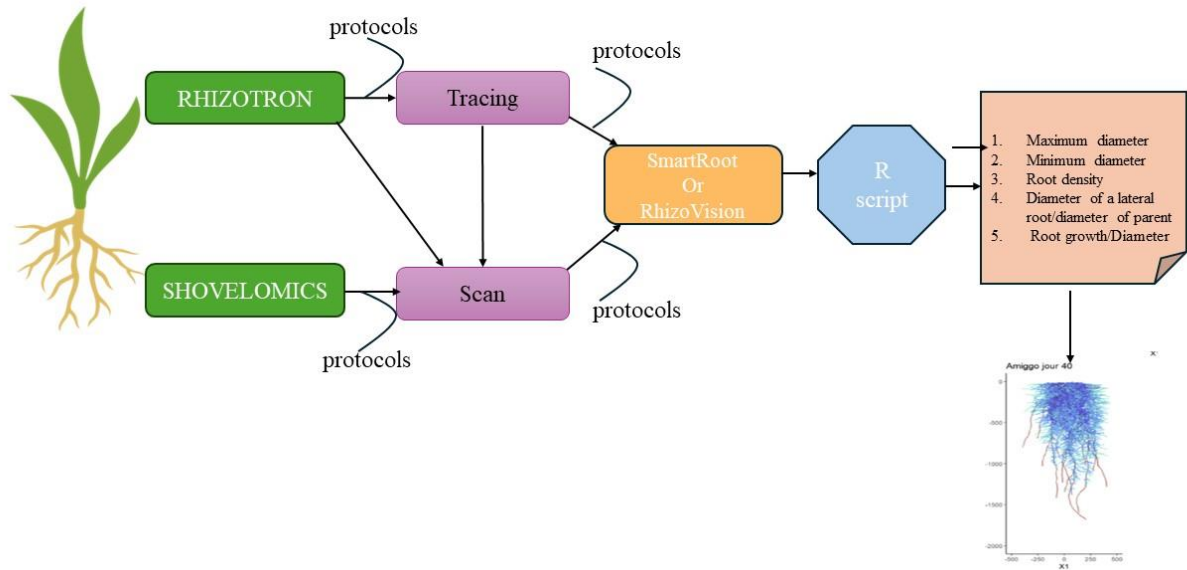


Figure 3 :Illustrated workflow for Utilizing the ArchiSimple Model

4. Methodology

4.1. The development of the R script

4.1.1. Data acquisition and their format

The data needed to test my script was retrieved from the [github](#) of a former student (Degives, 2023) who worked on the architecture of the sorghum root system. In addition, other students in the same laboratory were also working on analysing the architecture of the root systems of other plant species from which I was able to retrieve data. These were scanned and then the measurements were extracted using the SmartRoot software described above. As a reminder, the software allows the features to be exported in the form of a Comma Separated Value (CSV) file, which is just another way of presenting the information: rather than storing them in columns, CSV files store them separated by semicolons.

4.1.2. Creating functions

The functions created are designed to provide support for users. They do not involve analysis, but rather the extraction of the main parameters required for the model.

As this master thesis is intended to serve as a syllabus, each function used will be detailed in the results section. The statistical analysis software was used in version 2024.04.1+748.

4.2. Developing protocols

The creation of the protocols was based on a combination of bibliographical research (previous master theses) into several old works where manipulations had already been carried out in the past and where results had been collected, as well as on-site surveys. Through the latter, I was able to realize the importance of implementing this automated pipeline for students who had no idea where to start with their greenhouse manipulations. It was at this point that the pictures to illustrate the protocols were also taken.

4.3 Use of Artificial Intelligence Tools

In the preparation of this thesis, ChatGPT, an AI language model developed by OpenAI, was utilized to assist in the formulation and refinement of text. This tool was employed to ensure clarity, enhance the scientific tone, and aid in the efficient communication of complex ideas. After using this tool, I reviewed and edited the content as needed and take full responsibility for the content of the publication.

5. Results

5.1. Rhizotron protocol

5.1.1. Introduction and definition

Rhizotrons are specialized structures made to facilitate the study and visualization of plant root growth in a controlled environment. The term itself originates from the Greek words "rhizos" (root) and "tron" (instrument) (McGraw-Hill Dictionary of Scientific & Technical Terms). Basically, rhizotrons consist of chambers with transparent walls, allowing researchers to directly observe root development within the soil profile.

This root development observation tool is widely used at the UCLouvain's greenhouses.

This system offers several advantages, including:

- Non-destructive observation: Rhizotrons enable the continuous monitoring of root growth without disturbing the root system, unlike destructive methods such as excavation. This allows for a more comprehensive understanding of root dynamics over time.
- Real-time monitoring: Rhizotrons provide a real-time window into the root environment, enabling researchers to observe root responses to various stimuli, such as changes in soil moisture, nutrient availability, or microbial interactions.
- Quantitative data collection: Different methods can be employed within the rhizotron system to gather quantitative data on root growth.

These methods include:

- Tracing: This technique involves placing a transparent film on the rhizotron wall and manually tracing visible roots at regular intervals. This allows for the calculation of root growth rates over time.
- Root scanning and analysis: Digital imaging techniques, coupled with software like SmartRoot, enable the acquisition of detailed root system architecture data. This data can be used to quantify various root parameters, such as seminal root emission rate (erSem), primordial development time (PDT), and the relationship between growth rate and diameter (EL) (Pagès, 2014).

5.1.2. Schematic and dimensions

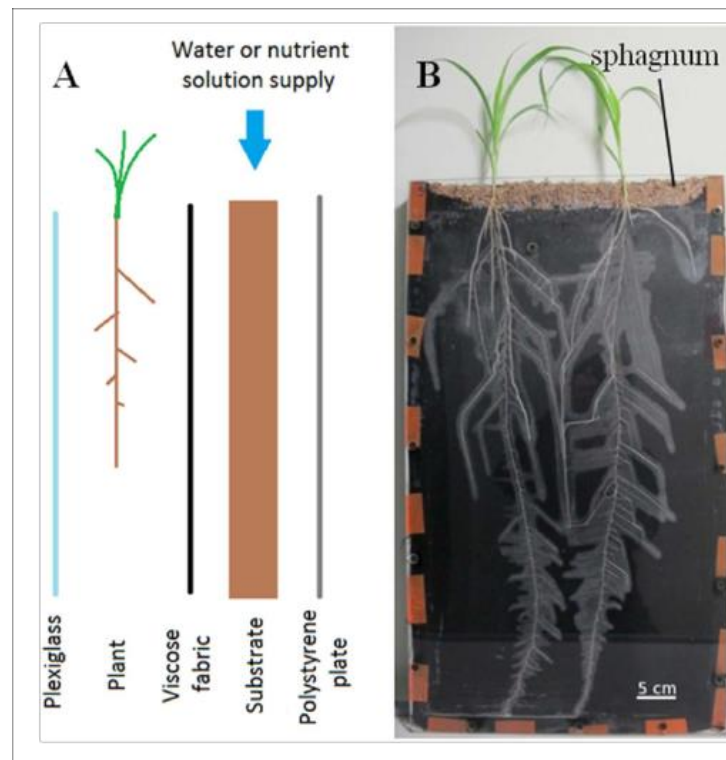


Figure 4: Diagram of rhizotron construction (Passot et al., 2016).

At UCLouvain, rhizotrons typically measure 20 cm in width and 40 cm in length. However, if the user has the option to choose the dimensions, it is crucial to consider several factors to select the appropriate rhizotron size.

The selection of the correct rhizotron size depends on the specific plant species under study. For species with relatively small root systems and slow growth rates, smaller rhizotrons may be sufficient to observe root development efficiently. Conversely, plants with extensive root systems or rapid growth rates require larger rhizotrons to accommodate their growth over time. Additionally, the duration of the experiment must be considered, as the size requirements will vary depending on whether the analysis spans a few weeks or several months.

Figure 4 provides a good illustration of how to arrange the various materials.

5.1.3. Material required

- Plexiglas sheet: They are therefore transparent and have a minimum thickness of 5mm.
- A wooden board
- Perlite-type substrate
- Sheet of filter paper
- Hoagland's solution
- Opaque plastic



Figure 5: Rizothron available from UCLouvain greenhouses.

5.1.4. Choice of substrate

The substrate must provide good aeration and drainage to avoid water stagnation around the roots. However, the substrate must also be capable of retaining the quantity of water required for proper root development. A 50/50 mix of perlite and vermiculite can be a judicious choice that meets the criteria. Perlite is a lightweight, porous material composed of volcanic glass. Its lightweight nature improves soil aeration and drainage without adding significant weight to the soil mix. Moreover, it has a neutral pH, making it suitable for a wide range of plants. It does not significantly alter the pH of the soil, allowing for more precise control over soil acidity or alkalinity (Storey, 2016). As for the Vermiculite, it has excellent water retention properties, absorbing and holding moisture in the soil. This helps prevent soil from drying out too quickly and provides a consistent water supply to plant roots. Like perlite, vermiculite has a neutral pH (Trees.com Staff, 2023).

5.1.5. Conditions

In order to carry out the seed growth experiment in rhizotrons, several steps need to be taken into consideration. To begin with, it is important to choose a suitable location where the climate is conducive to the growth of the specific seeds being studied. In cases where the student or PhD student carries out his or her manipulations via the university, greenhouses provide an ideal environment for many types of seeds by simulating several desired climates.

To reduce the risk of fungal growth in the system, adequate ventilation is essential to reduce excessive humidity. While controlling ventilation can be problematic, managing humidity levels through watering practices can help maintain an optimal environment for seed growth.

Keeping equipment clean is essential. Ethanol is an effective disinfectant, as it damages microbial structures and inhibits their growth (Salaspuro, 1997). It is therefore advisable to thoroughly clean all rhizotron components with ethanol to ensure a sterile environment. As well as keeping the equipment clean, disinfecting the seeds with a 5% bleach solution can considerably reduce the risk of contamination and improve germination success (Normand et al., 1995, Chapter 6). After soaking the seeds, it is important to rinse them thoroughly before placing them in the rhizotron.

In extreme cases where fungal growth persists, the use of biological fungicides may be necessary. However, it is crucial to apply these fungicides before using the rhizotron to effectively combat fungal growth.

To avoid phototropic interference with root growth, it is recommended to cover the section of the rhizotron containing the visible roots. This maintains an undisturbed environment for root development.

Finally, maintaining good hydration is essential for seed growth. For this reason, it's advisable to water the seeds every other day with a syringe, at a rate of 20 ml each time.

5.1.6. Setting up the rhizotron

- Soak the filter papers in the Hoagland solution (see Appendix), the substrate is also mixed with the solution.
- Fill the wooden board with the substrate.
- Place the sheet of filter paper on the substrate.
- Place the seed on the filter paper, approximately one centimeter from the edge.
- Close the container with the Plexiglas plate.

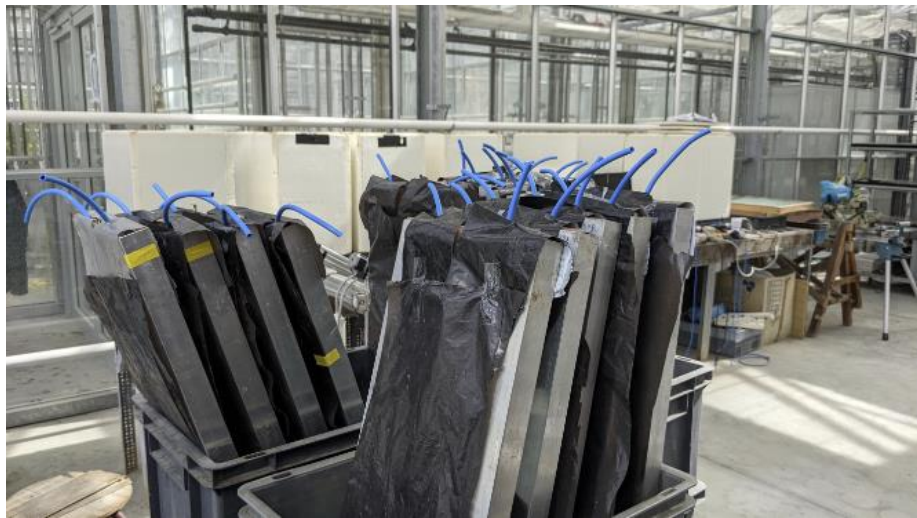


Figure 6: Rhizotrons in greenhouses (HenrichThuet,2023).

5.1.7. Root preparation for scanning

- Select the plant whose root system you wish to digitize.
- Carefully remove the plant from the soil or substrate, preserving as much of the root system as possible.
- Gently clean the roots to remove excess soil or substrate, taking care not to damage the roots.
- Cut at the collar to separate the stem system, which you will keep carefully, from the root system.
- If the root system is too large, it can also be divided into several parts for scanning.
- The root system can be scanned directly within the rhizotron.



Figure 7: Root developed at end of rhizotron experiment. (HeinrichThuet,2023).

5.1.8. Tracking lines

In setting up the rhizotron, transparent paper (Figure 8) is added to its surface to trace the evolution of the roots using indelible markers.

The ArchiSimple model incorporates dynamic parameters such as the seminal root emission rate (erSem), adventive root emission rate (erAdv), primordial development time (PDT), and notably, the slope of the relationship between growth rate and diameter (EL).

The required materials are simple, typically involving several colored indelible markers to differentiate between various root types, such as seminal and lateral roots. Regular tracing is essential, with additional dots added beside different roots on a daily basis to monitor their growth progression. The growth parameter will then be calculated from the tracks and the mark function on SmartRoot. For ease of use, the marks can be made on the same day as watering, i.e. every other day.

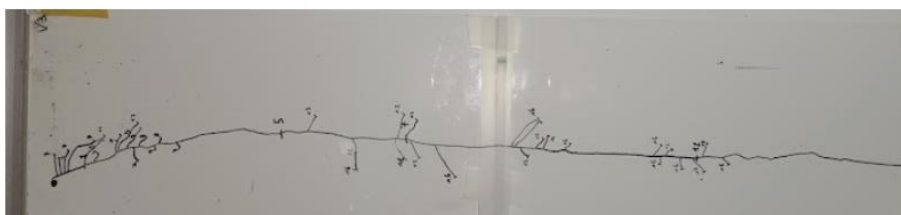


Figure 8: Root growth tracing (HeinrichThuet,2023).

5.2. Shovelomics protocol

Shovelomics is a term used in plant science to describe a methodology focused on the comprehensive and high-throughput phenotyping of roots. It involves the systematic excavation and analysis of root crowns from soil using shovels or similar tools. The objective of shovelomics is to understand the genetic and physiological basis of root traits and their adaptation to different soil conditions (Trachsel, 2011). This method is essentially a variation of the modified monolith approach. Shovelomics focuses on studying root architecture by analyzing excavated root systems, while a monolith in plant science typically refers to a sample of intact soil used for studying soil properties and root distribution within the soil profile (Freschet et al., 2021).

5.2.1. Materials required

- Shovel or trowel
- Collecting tray for excavated soil
- Soft brushes
- Water containers for cleaning roots

5.2.2. Select a plant of interest

5.2.3. Digging

To dig up the selected plant, the first step is the use of a shovel or trowel. Ensuring that it is deep enough to expose the roots without damaging them. It is essential to maintain a certain distance from the base of the plant so as not to restrict examination of the roots. Conversely, it is important to avoid digging a hole that is too wide, as this could damage the root structure and lead to the breakage of certain parts. The appropriate distance from the stem varies according to soil texture, but in general, leaving around 25 cm around the stem strikes a balance, providing adequate visibility of the roots without risking damage to them through excessive excavation (Penn State College of Agricultural Sciences, n.d.).

5.2.4. Root cleaning

Once the plants are harvested, they need to be cleaned (Figure 9). To do this, a washing table is used, consisting of a very fine sieve that removes almost all the soil particles using a powerful jet of water. The key is to get rid of any soil particles that might interfere with the scans of the root systems. The process is made easier by leaving the roots to soak in water for at least an hour.



Figure 9: Root cleaning set-up.

5.2.5. Results

For this precise method, it will not be possible to make a regular trace as the roots will be growing underground. One way of obtaining measurements that can then be used for future analysis is to scan them.

5.3. Root scanning protocol

After the plants have matured in the field, they are excavated according to the shovelomics protocol. To acquire the remaining static parameters, such as root diameters and the ratio of diameters between "daughter" roots and their corresponding "mother" roots, one method involves scanning the roots.

5.3.1. Material required

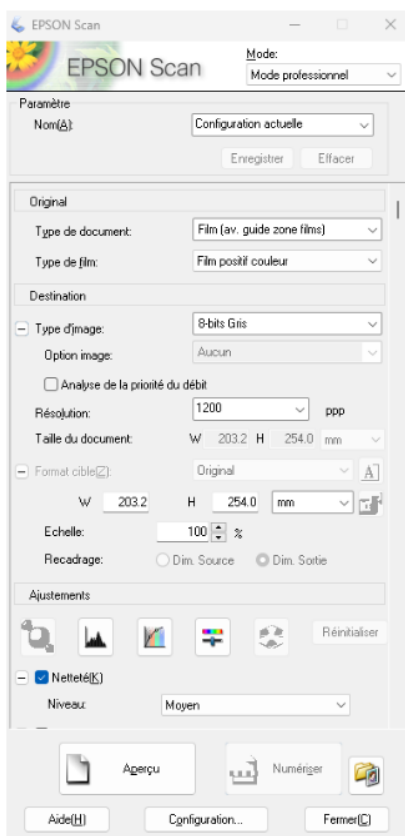
- Epson V850 Pro Mini scanner or equivalent.
- Computer with Epson scanning software installed.
- Plants with root systems that you wish to scan.
- A tray.
- Demineralized water.

5.3.2. Root system scan

- Prepare a transparent tray filled with water.
- Carefully place the roots in the tray, ensuring they are fully submerged in water. The presence of water facilitates the spreading of the roots.
- Once the roots are positioned in the tray, place the tray on the scanner bed.
- Ensure that the roots are arranged in a way that allows for clear and accurate

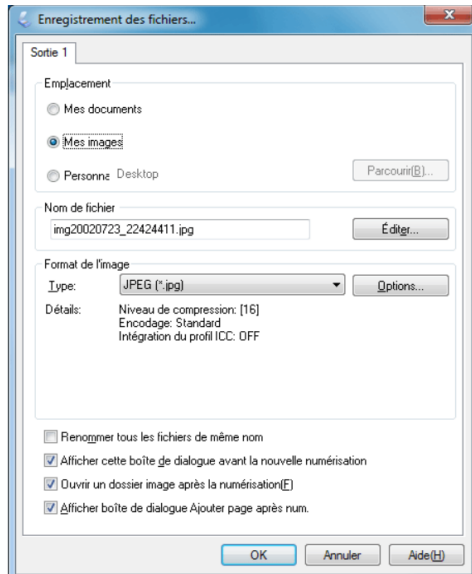
scanning. This involves positioning and organizing the roots to optimize the quality of scanned images and facilitate accurate measurement and analysis. Overlapping roots can obscure each other in the scanned image, making it difficult to distinguish individual roots and accurately measure their characteristics. Therefore, arranging roots to minimize overlap helps ensure that each root is clearly visible and can be analyzed independently.

- Switch on scanner and computer.
- Open the Epson scanning software on your computer.
- Select the appropriate scan settings, including resolution and file format (Figure 10).
- Start scanning.
- You can then save the file by clicking on Save files to the right of the Scan button.
- The File Save window appears. Files are generally saved in TIFF format, a file format created to exchange data between numerous



programs (Figure 11).

Figure 10: Scan settings (Degives, 2023)



- For information on the scanner itself, please click [here](#) (Guide de l'utilisateur Perfection V800 Photo/V850 Pro).

Figure 11: File Saving EPSON V850 Pro (Guide de l'utilisateur Perfection V800 Photo/V850 Pro)

5.4. Scan Analysis

5.4.1. SmartRoot: A semiautomated companion for dynamic root system analysis

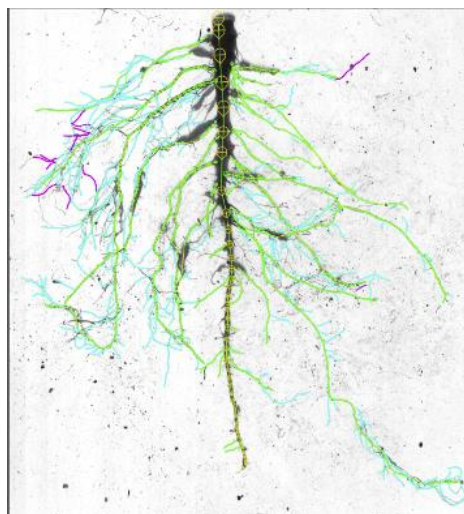


Figure 12: SmartRoot image.

SmartRoot, an ImageJ-based software package developed by Lobet et al. (2011), offers robust capabilities for quantifying root growth and complex architecture (Figure 12).

Users can access a helpful tutorial titled "Root phenotyping: measuring roots with ImageJ SmartRoot plugin" on YouTube, created by user [ChéFaire](#).

SmartRoot facilitates efficient root system analysis, switching from conventional imaging techniques to dynamic parameters such as growth rates. Whereas manual software requires laborious manual drawing, SmartRoot's semi-automatic approach combines automated algorithms with user intervention, providing a balance between efficiency and accuracy.

The software organizes information into layers (Figure 13): the original image, root morphology data in vector format, spatial connections between root segments, and user annotations along roots for enhanced analysis. This layered approach provides a comprehensive representation of root shapes, structures, and relationships within the root system.

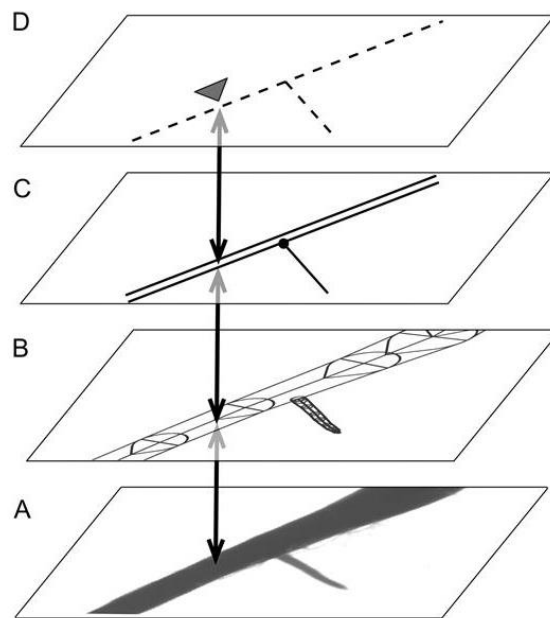


Figure 13: The 4 layers containing the information. A is the source image, B, the morphology, C, Topology and finally, D, the annotations (Lobet et al., 2011).

The software features an advanced function that semi-automatically traces individual roots in images. Each time a user clicks with their cursor on a root in the image, this tool springs into action. Using an intelligent algorithm, SmartRoot identifies the centerline of the root near the clicked position. It then carefully draws a segmented line along the midline of the root, progressing from its tip to its base. Throughout this process, SmartRoot estimates the thickness of the root at each point on the segmented line. This information is essential for accurately representing the shape of the root.

Without going into too much detail, the algorithm systematically analyses pixel values along a curved path to trace root structures in images. It first searches for the direction of the root from the current point, adjusting the search angle and distance if necessary. When a potential next point is located, the algorithm refines its position by examining numerous points along the edges of the root. It assesses the distance between these edges to determine the thickness of the root and any potential contact with adjacent roots. This process ensures accurate tracing of root structures while minimising errors.

The algorithm adds newly created nodes to the segmented line, building it until it stops. Its parameters are determined empirically and can be adjusted for specific images. As users can modify the newly created roots by simple drag-and-drop actions on the nodes, intuitive adjustments can be made without modifying the source image. Multiple roots can be drawn simultaneously by selecting them using a line-drawing tool.

SmartRoot establishes topological relationships by connecting lateral roots to their parent roots, making it easier to calculate and monitor root development. It relies on an algorithm to detect and trace lateral roots, discriminating between them on the basis of size, position and branching angles.

An interesting and important feature of the software is its ability to analyse time-lapse image sequences, i.e. a series of photos taken over time (Figure 14). This allows the user to study root growth and development. Among ArchiSimple's parameters, measuring growth is very important and is acquired using this function. SmartRoot can link information from different images, such as annotations or root branches, to specific points along the root. It can then display all this information in a single image, usually the last in the series. This makes it easy to see how roots evolve over time and to analyse the image as a whole.

To do this, SmartRoot uses what is known as the $[r, d]$ coordinate system. Here, 'r' tells us where we are on the root, usually measured as a percentage from the end of the root, while 'd' tells us the diameter of the root at that point. This system allows SmartRoot to process images taken at different times, even if they don't overlap perfectly. So, whether the roots are growing in a liquid or in the air, SmartRoot can trace and follow them in different images. It offers tools to facilitate this process, such as the ability to see the same root in several images at once.

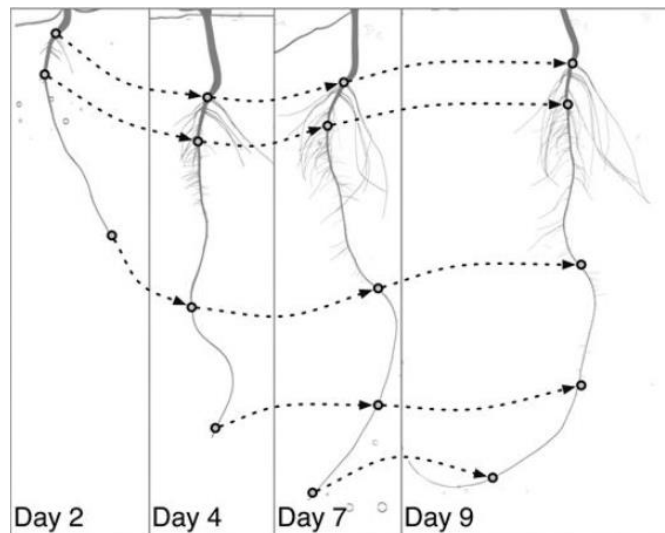


Figure 14: Time-lapse image sequences of plants (Lobet et al., 2011).

Another advantage of SmartRoot is that it can analyse root images taken with different types of cameras or scanner. Of course, the best results come from high-resolution images taken with a flatbed scanner, but this method can be slow and is not ideal for collecting large-scale images. SmartRoot can, however, analyse lower quality images, such as those taken with a camera, as long as the roots are visible and at least two to four pixels wide. Background noise and contrast are generally not a problem. What's more, SmartRoot can process images from different configurations, such as aeroponics or Petri dishes. It reads common image file types such as jpg, gif, tif and bmp, and processes them in greyscale, where the roots appear darker than the background. If required, colour images can be converted to greyscale and the software can also adjust the contrast.

The 'global root data export' function displays data on individual root structures when the root nodes export function provides an overview of individual nodes within the root system. In addition, the All Marks function provides full details of all the marks in the image. Alongside the marks added by the user, SmartRoot also exports an origin mark (indicating the start of the root) and a length mark (indicating the end of the root). Diameter and angle measurements are captured at the position of each mark.

Moreover, the 'root length density' export function is specifically designed for plants grown in rhizotrons. It quantifies the root length density (cm of roots / cm³ of soil) in a user-defined area.

The 'growth rate' export function is tailored for time-lapse monitoring of plant growth in rhizotrons. It facilitates the export of root growth data based on the marks placed on the roots.

The software provides various functions, such as Trace Root or Trace Lateral, to record architectural information effectively. To utilize these features, ensure the plugin is installed on ImageJ and utilize trace and mark tools. If necessary, invert colors to enhance root visibility. Draw roots using Alt+left click (Windows) or Option+left click (Mac).

When prompted to enter the root identifier, designate the main root as R1 and lateral roots with green tracer labels like lat_1, etc.

Upon clicking the Root list window, the main R1 root and its associated lateral specifications, including length, volume, average diameter, area, and projected area, are displayed. Once tracings are completed and desired measurements appear, use the Data transfer window to export data (Root name, length, vector_length, surface, volume, root ontology) in CSV format.

To summarise what we have seen, SmartRoot is capable of extracting root length and diameter, root insertion angle, lateral density and growth topology, which are listed in table 3 along with their significance. It also offers the possibility of directly extracting more global root data in a CSV (Comma Separated Values) file.

Table 2: Measured traits and their explanation (Lobet et al., 2011)

Measured traits	Explanation
child density	the ratio between the number of child roots and the length (cm) of the parent root within the branching area.
diameter	calculated by measuring the diameter of each node along the root and then finding the mean value.
first child	when a new lateral root is formed, it will be designated as a child of the chosen parent root.
distance_ from_ base	the location of a node along the root in relation to its base.
distance_ from _apex	the location of a node along the root in relation to its apex.
growth	This indicates how much the root has grown since the previous measurement was taken.
image	Image name
insertion angle	insertion angle on the parent
insertion first child	refers to the location of the first child root branching off from the base of the main root.
insertion last child	refers to the location of the last child root branching off from the base of the main root.
insertion position	This describes where a new root attaches to the parent root, measured from the base of the parent.
last child	name of the last child
length	root length
n_child	The number of child roots
parent	parent identifier
root	root identifier
root_ name	Root name
root_ontology	the categorization of a root based on its classification within an ontology system.
root_order	the level of the root's branching hierarchy, with 0 representing the first level of branching.
theta	the angle at which a node is positioned along the root, measured in radians.
value	value of the mark along the root
x and y	the horizontal (x) and vertical (y) position of the center of a node along the root, measured in centimeters.

5.4.2. RhizoVision: Root System Analysis through Full Automation

The C++ program RhizoVision Explorer (Seethepalli and York, 2020) makes use of the C++ library cvutil to improve OpenCV's features. This library helps with a variety of tasks, including adjusting image contours as well as creating distance maps.

Moreover, it provides a user interface (UI) and allows plugins to expand it.

To begin using RhizoVision Explorer, drag and drop an image of a plant root into the window or use the file menu to load it. It can work with a number of image types, including PNG, JPEG, BMP, and TIFF, however for color images, it only utilizes the red channel. The Analysis Options window, located on the left (Figure 15), is where you change the image processing and result display settings.

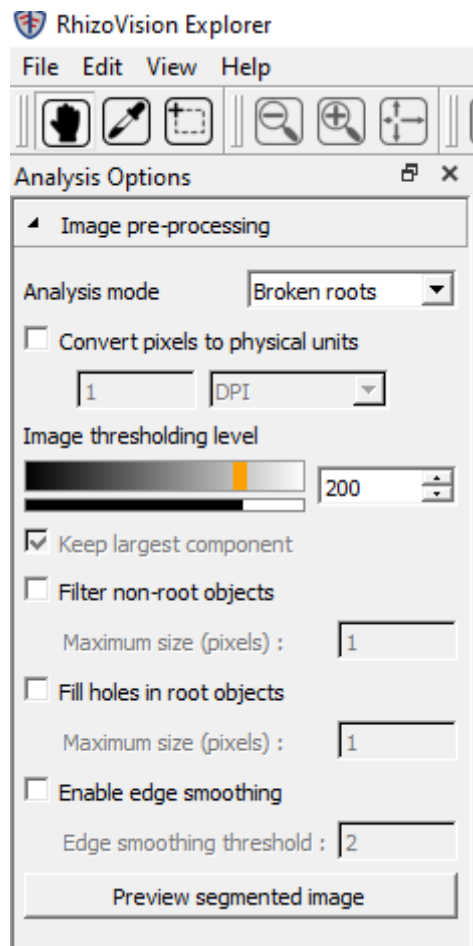


Figure 15: The Analysis Options window.

After adjusting these settings in accordance with the research, the software enables the user to preview the modifications and initiate the analysis. When the 'run analysis' button is clicked, the processed image appears in the window's center and features are extracted from the root image.

Although it provides a mode for evaluating entire roots with additional properties including convex hull (a geometric shape that outlines the outermost points of the root system), root width, depth, and angles, it analyzes broken roots by default.

If necessary, it's possible to translate pixel measurements into tangible measures like millimeters or inches. In addition to standard settings such as thresholding and color inversion, the application can fill in minor gaps in the roots and filter out non-root objects from the background.

Once the settings have been set right, users can even use the batch mode to analyze numerous images at once. It saves the output for each image in a folder with the same settings applied.

The main skeleton of the roots is found by measuring distances between points. If this skeleton isn't fully connected, a method combining the Euclidean distance transform and the steepest ascent algorithm is used to fill in the gaps and complete it.

After skeletonizing the segmented image, the root's structure is identified by finding its topological branch points, end points, and the segments between them. End points, or root tips, are pixels with only one neighbor, while branch points have three or four neighbors. This structure is stored as a copy of the skeletal image with pixels encoding their type.

A method, called 'root pruning,' reduces the number of short, invalid lateral roots without changing the shape of the segmented root image. It works by evaluating root segments starting from branch points and ending at tips. If a lateral root is too short, it's deleted from both the topological and skeletal images. This process repeats iteratively until no invalid lateral roots remain, ensuring accurate analysis results.

Features like surface area and volume are calculated assuming each pixel in the skeleton is a cylinder. As with SmartRoot, Table 4 lists all the features obtained from the software and their simpler explanations. (Seethepalli et al., 2021)

Table 3: List of all RhizoVision's features and their explanations (Seethepalli et al., 2021).

Measured traits	Explanation
Number of root tips	The number of root tips is the count of pixels in the recognized root structure that are connected to just one other pixel.
Total Root Length (px, mm)	The total length of all the straight lines connecting the skeleton pixels throughout the entire root structure in the plant root picture.
Network Area (px ² , mm ²)	The total number of pixels in the segmented image.
Median, Average, Maximum Diameter (px, mm)	At each skeleton pixel, the distance transform value represents the radius, which is then doubled to get the diameter. The average, middle, and largest diameters are then computed using all these skeleton pixels.
Perimeter (px, mm)	The total length of all the straight lines connecting the contour pixels throughout the entire segmented image of the plant root.
Volume (px ³ , mm ³) and Surface Area (px ² , mm ²)	The radii of each skeleton pixel help calculate the volume at each pixel. This is done by multiplying the pixel's length by the root's cross-sectional area. Similarly, for surface area, the length of each pixel is multiplied by the root's circumference. Then, the total volume and surface area are found by adding up all the volumes and surface areas from each pixel.
Computation Time (s)	The time it takes to analyze a plant root image.
Root Length, Projected Area, Surface Area and Volume histograms	Each pixel in the root structure is measured for its length, area on the image surface, surface area, and volume. These measurements are then grouped based on the diameter ranges chosen by the user.
Number of Branch Points	The number of branch points refers to pixels in the identified root structure that are connected to at least three neighboring skeletal root segments.
Branching Frequency (px ⁻¹ , mm ⁻¹)	The count of branch points per unit length of the root.
Median and Maximum number of roots	For each row in the segmented image, a horizontal scan is done, counting how many times the image transitions from a background to a root pixel. This list of transitions is organized, and the median and maximum counts of roots are identified.

Depth, Maximum Width (px, mm)	The maximum vertical and horizontal distances the root crown reached during imaging are recorded as Depth and Maximum Width, respectively.
Width-to-Depth Ratio	The ratio of the maximum width to the depth is called the Width-to-Depth ratio.
Convex Area (px ² , mm ²) and Solidity	The Convex Area refers to the area of the smallest convex shape that can cover the entire root crown system. Solidity is the ratio of the Network Area to the Convex Area.
Lower Root Area (px ² , mm ²)	In a root crown image, the skeletal pixel with the largest radius is identified. The area of the root system beneath this pixel is then noted as the Lower Root Area.
Holes and Average Hole Size (px ² , mm ²)	Holes are spaces between roots in a segmented image. Their count and average size are measured.
Steep Angle Frequency, Medium Angle Frequency, Shallow Angle Frequency and Average Root Orientation	In a 40 × 40-pixel vicinity around each skeletal pixel, the coordinates of all skeletal pixels in that area are collected. Their angular orientation is then calculated and grouped into bins of 0–30°, 30–60°, and 60–90°. The frequencies in each bin are noted as Steep, Medium, and Shallow Angle Frequencies. The average of these orientations is recorded as the Average Root Orientation.

RhizoVision Explorer incorporates various features aimed at enhancing the software's user experience. One of these features is the Region of Interest (ROI) system, which is referred to as the ROI Manager. It is made possible to access the ROI tool from the toolbar to create multiple ROIs. The ROI Manager enables users to assign custom names to selected ROIs, manually adjust their XY coordinate position, width, and height, and maintains a list of ROIs within the image. Each ROI defines a specific area within the image, allowing the analysis of roots contained within that region. The results of this analysis are reported separately for each ROI, with a dedicated column indicating the name of the ROI. Additionally, users can save and load ROIs as annotation files, and these selections are retained for batch processing.

The biggest advantage of the software is the time it saves the user and the ability to analyze large datasets efficiently, enabling rapid phenotyping of root characteristics across multiple samples or experimental treatments.

In addition, data such as maximum diameters can only be obtained from other software, such as SmartRoot, through statistical analysis using software such as R.

5.5. ArchiSimple Model: A deep dive into the model and its parameters

It is crucial to understand how plants interact with the soil through their root system, and root system architecture models play a key role in this. However, these models face a number of challenges. For example, while they allow us to understand where roots grow and what their structural properties are, they are often adapted to specific plant species or growth stages. In addition, integration with broader plant or ecosystem models is lacking in many cases. Finally, some root system architecture models are too complex, making them difficult to use and interpret.

To address these challenges, a simpler model known as "ArchiSimple" was developed (Pagès et al., 2014). This model focuses on the fundamental aspects of root system architecture dynamics and aims to facilitate understanding and application to a variety of plant species and environmental conditions. By simplifying the modelling approach and reducing the complexity of parameters, ArchiSimple improves usability and facilitates wider applications in research and agricultural practices.

It is designed to represent root systems in three dimensions: by width, depth and growth over time. This model shows the different ways in which roots grow and develop from one plant species to another, taking into account how they interact with the environment. Despite its complexity, ArchiSimple aims to keep things simple by using a basic set of parameters to accurately capture these processes. It is built in C++, a programming language known for being powerful, versatile, efficient, robust and extensible. The model parameters consist of static and dynamic elements, focusing primarily on root diameters and growth rates.

ArchiSimple can be pictured as a digital representation of plant roots (Figure 16), using discrete segments and meristems—regions where cell division occurs—to simulate growth dynamics.

Time in the model is divided into daily intervals, during which it executes algorithms mimicking essential root growth processes.

For a better understanding of the logic behind the model, the various development processes will be briefly discussed in the following subsections.

a. Emission

To simulate the growth of additional roots, it is assumed that the number of roots produced increases steadily over time (represented by the slope, ER, in days⁻¹) until it reaches a maximum value. In plants characterised by a taproot, this maximum value is generally 1. However, in monocots, for example, multiple primary roots emerge from the base of the shoot. Each newly emerged root is then assigned a tip diameter (parameter Di, mm), which determines its rate of elongation after emergence. (Pagès et al., 2014)

b. Elongation

When a root starts growing, it goes through a stage called primordium for about 5 days. After this, it becomes mature. Many root models assume there's a gap between when the root starts and when it actually emerges. (Pagès et al., 2014)

The speed at which the root tip grows depends on its diameter (thickness) and how old it is. If the diameter is above a certain minimum and the root is young enough, it grows. Otherwise, it doesn't. There are three factors involved here: EL, which is how much the root elongates for each millimeter of diameter it has (measured in mm mm⁻¹ day⁻¹), GD (equation 1), which is how long the root grows (calculated from GDs, being then a growth duration parameter [day * mm²] and Dmin, which is the smallest diameter a root can be before it stops growing.

$$GD = GDs * D^2 \quad (1)$$

The actual growth rate of the root is the potential growth rate (PER, in mm day⁻¹, equation 2) times how satisfied the root is (SC), and the reduction caused by the soil where the root is growing.

$$PER = EL * D \text{ if } D > D_{min} \text{ and } Age < GD, PER = 0 \text{ if } D \leq D_{min} \text{ or } Age \geq GD \quad (2)$$

The time it takes for a root to grow is also linked to its thickness. This is worked out using an equation where the thickness of the root tip is squared. This works well for many plants.

Roots don't always grow in straight lines because they encounter things in the soil and respond to gravity. The direction they grow affects how the whole root system looks. Their direction is calculated based on where they started, some random changes caused by the soil, and whether they're being pulled down by gravity or growing sideways (Pagès et al., 2004).

c. Branching

New lateral roots begin to form one after the other, starting near the tip of the main root and moving towards the base. They're spaced out regularly, with the distance between them determined by a parameter called IPD, measured in millimeters. These lateral roots begin to grow after a certain amount of time, as mentioned earlier.

The number of lateral roots that form can also be influenced by the conditions of the soil where the main root is growing. Each new lateral root emerges with a diameter linked to that of its parent root.. This is figured out using two factors, RDM and CVDD, without units. The diameter of the lateral root is chosen randomly from a normal distribution, where the average (mean) diameter is the diameter of the parent root multiplied by RDM. The standard deviation is calculated by multiplying the mean by another factor, CVDD (Pagès et al.,2014).

d. Decay and abscission

Decay begins when the time since root growth stops exceeds the root's lifespan. The lifespan (LD) is figured out using this formula:

$$LD = LDC * D^2 * RTD \quad (3)$$

where LDs represents a model parameter, D is the diameter of the root tip, and RTD stands for root tissue density, also a model parameter. Once a root dies, it typically falls off unless it has any living side roots. If there are living side roots, the dead root stays attached to its main root. It should be noted that environmental factors likely to affect root lifespan are not taken into account in this simplified model.

e. Radial growth

The cross-sectional area of a root at a certain point is the sum of many small sections, each representing a part of the root system further away from that point. So, when a root starts to grow, it adds to the width of the roots it's connected to, based on its own cross-sectional area. The proportionality coefficient in this model is called SGC. It's been calculated for various plant species, with results close to 1 for many. However, for monocotyledonous species, this coefficient is 0, because their roots don't undergo radial growth (Pagès et al., 2014).

Once the simulation is complete, the model offers the option of extracting additional relevant parameters. All the segments of the root system, with their characteristics such as length, diameter, branching and age, are recorded in an output file.

From this data, additional information such as depth, total length and surface area occupied by the root system can be obtained (Pagès et al., 2014).

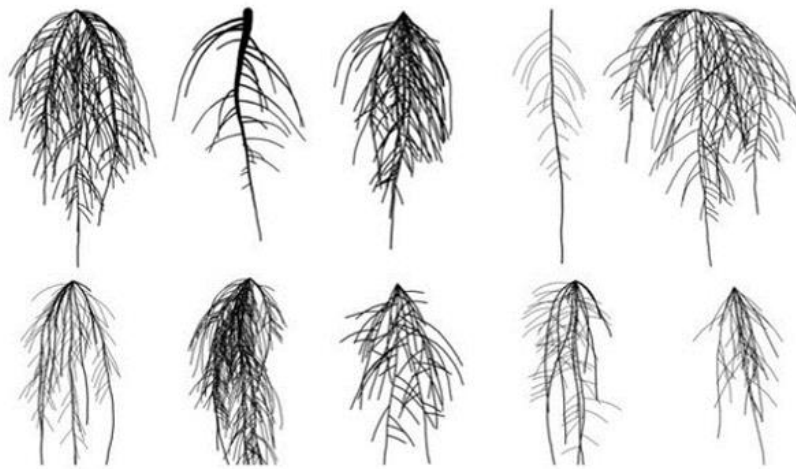


Figure 16: Root images obtained with ArchiSimple (Pagès et al., 2014).

5.5.1. ArchiSimple model parameters

Table 4: ArchiSimple parameters and their units.

Parameters	Abbreviation	Unit
Simulation time	simtime	day
Seminal root emission rate	erSem	day^{-1}
Proportion of maximum diameter for seminal roots	dSem	/
Maximum number of seminal roots	maxSem	/
Age at which adventitious roots emerge	ageAdv	day
Maximum distance from base for adventitious roots	distAdv	mm
Adventitious root emission rate	erAdv	day^{-1}
Proportion of maximum diameter for adventitious roots	dAdv	/
Maximum number of adventitious roots	maxAdv	/
Radial growth coefficient	SGC	/
Minimum diameter	dmin	mm
Maximum diameter	dmax	mm
Slope of relationship between growth rate and diameter	EL	$mm.mm/day$
Type of gravitropism	TrT	/
Gravitropism intensity	Trint	/
Growth duration coefficient	GDs	day/mm^2
Primordias development time	PDT	day
Inter-branching distance	IPD	mm
Probability of emergence of a lateral on an axis of diameter dmax	pdmax	/
Probability of emergence of a lateral on an axis of diameter dmin	pdmin	/
Slope of the ratio of the child diameter to the parent diameter	RDM	/
Coefficient of variation of branch diameter	CVDD	/
Densité des tissus racinaires	TMD	$g.cm^3$
Life expectancy coefficient	LDC	$day.mm.mg^{-1}$

5.5.2. An example of application

Plant species are distinguished by the way they grow underground. We saw a few sections earlier that there are two main categories: primary root systems, also called ‘taprooted’, which develop by elongation and branching of the initial root, and secondary root systems, ‘fasciculated’, characterised by a bundle of roots that extend horizontally in all directions. The ArchiSimple model can simulate these different types of root system, as shown in Figure 17 (Pagès et al., 2012).

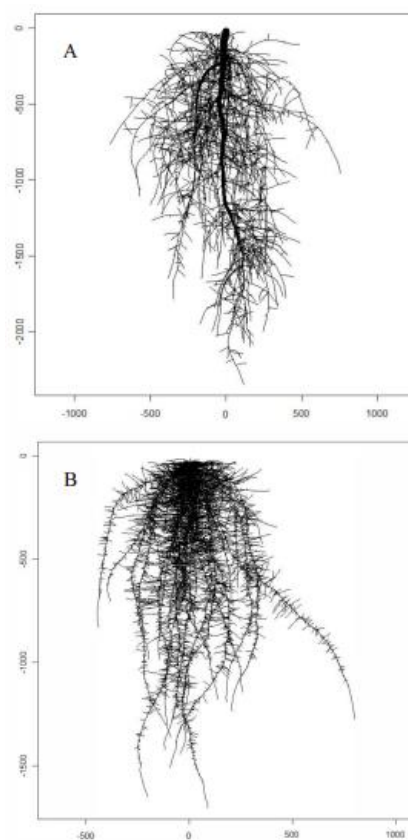


Figure 17: Two examples of simulated root systems. The first one being a primary root system (A) and the second, secondary root system (B) (Pagès et al.,2012).

But that's not all, ArchiSimple is also capable of representing and analysing the interactions between the soil and the roots as they grow. In agriculture, soil compaction is a recurring problem that can lead to the formation of a plough pan and thus degrade roots. In this situation, root elongation tends to be reduced. Figure 18 (Pagès et al., 2012) shows the simulation of roots in this type of compacted environment.

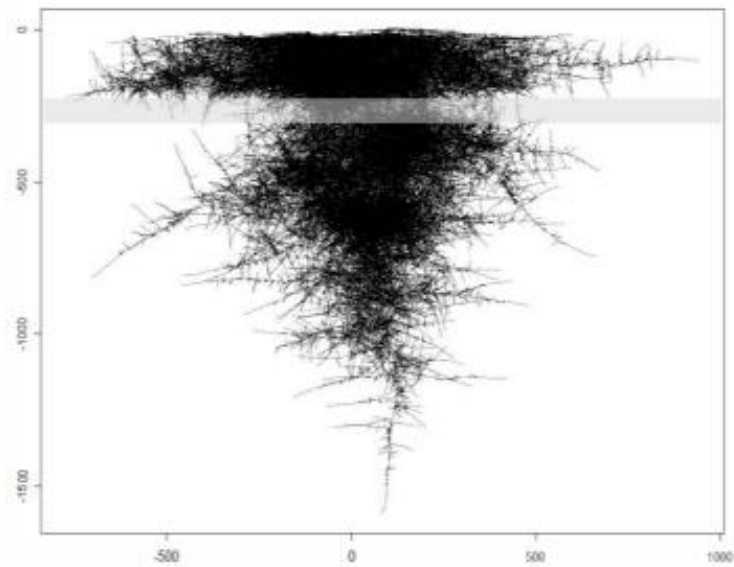


Figure 18: Modeling the impact of a plough pan on the growth of root systems (Pagès et al.,2012).

So, ArchiSimple helps us understand how a plant's root structure and its ability to change affect the movement of substances between the plant and the soil. Indeed, this model was created to put together a digital version of a plant's root system with its structure. It's used to study how different genes and the environment affect each other in a complex system. ArchiSimple represents the root system as a growing set of root parts, each with its position, age, and shape. This method lets us simulate processes that change over time, like how roots release carbon, absorb phosphorous, or take in water.

5.6. Data analysis

5.6.1. Detailed explanation of the functions in the script

5.6.1.1. Exploring the Key Parameters in ASR Characterization

In a study by Pagès and Kervella (2018), 60 species were sampled across two different environments with homogeneous soil conditions. Five key root architecture traits were measured for each species and environment: extreme tip diameters (D_{min} and D_{max}), relative diameter range (D_{range}), mean inter-branch distance (IBD), and the slope of the linear relationship between parent and lateral root diameters ($DIDm$). Analysis of interspecies variations in these traits revealed significant patterns, demonstrating distinct root characteristics among species and underscoring the importance of these traits for understanding root architecture across different environments. Consequently, I chose to focus on these five parameters for the script. However, as D_{range} is not a required input parameter for the ArchiSimple model, it was not included in the R script.

Before providing a more detailed analysis of the script, it is essential to understand these parameters and their relevance. The minimum diameter (D_{min}) reflects the fineness of the numerous fine roots primarily involved in absorption. Developing very fine roots (low D_{min}) is a key strategy for enhancing the soil-root exchange surface while minimizing costs. This strategy is particularly advantageous as the finest roots have a simple structure with low tissue density (Pagès and Kervella, 2018). The maximum diameter (D_{max}) often corresponds to the largest roots, which penetrate deep into the soil, expanding the soil volume explored by the plant. These larger root tips play a crucial role in determining the overall availability of soil resources. The depth to which the roots extend often indicates water availability for the plant. Larger diameter tips also facilitate penetration into dense soils, providing an advantage in soil exploration. It is important to note that the maximum diameter does not necessarily correspond to the longest roots. Instead, it refers to the thickest root tips, which are crucial for penetrating dense soils and accessing deeper soil layers.

Mean inter-branch distance (IBD) is a significant measure of root system efficiency in utilizing soil. It indicates the average distance between lateral roots growing on main roots. This measure helps determine root density within a given soil volume. To calculate IBD, the distances between lateral roots on main roots, whose diameter is greater than a certain threshold, must be measured. This threshold is calculated as half the sum of the smallest (D_{min})

and largest (D_{max}) diameters of the main roots. Essentially, IBD indicates how far apart lateral roots grow on larger roots. The slope of the linear relationship between the diameter of a lateral root and its parent root, referred to as DID_m , illustrates how lateral root diameter varies with parent root diameter. This value is obtained by calculating the slope of the linear regression between the diameters of daughter and parent roots. This relationship corresponds to the RDM parameter in the ArchiSimple model (Pagès, 2012).

Understanding this hierarchy between mother and lateral roots is crucial for optimizing root system efficiency, minimizing root overlap, and reducing competition for soil resources. By spacing lateral roots appropriately, plants ensure efficient soil utilization.. The relationship between mother and daughter root diameters can be graphically represented (Figure 19), as done in a previous master thesis (HeinrichThuët, 2023). In this graphical representation, one axis indicates the size of the mother root, and the other indicates the size of the daughter roots. Each point represents a pair of values: the size of a mother root and the size of its corresponding daughter root. A diagonal line with a slope of 1 indicates points where daughter roots are the same size as mother roots. Most points below this line indicate that daughter roots are thinner than mother roots, which is typically the case. The regression line on the graph shows the general trend in daughter root diameters relative to mother root diameters. An upward-sloping regression line suggests a positive relationship between mother and daughter root sizes, while a downward-sloping line suggests the opposite. A horizontal line indicates no consistent change in daughter root diameters with changes in mother root diameters.

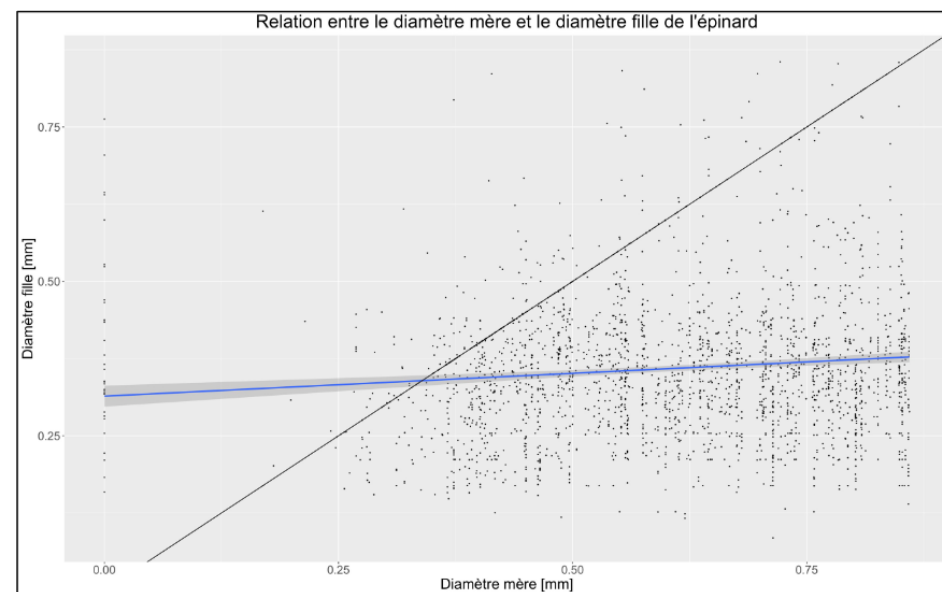


Figure 19: Spinach, its relationship between the diameter of mother roots and daughter roots (Heinrich-Thuët, 2023).

The coefficients of variation for daughter diameters (CVDD) were determined by analyzing the linear relationships between the standard deviations and the means of daughter root diameters, categorized based on their parent root diameter. In essence, the CVDD quantifies the extent of variation in the size of daughter roots relative to their parent roots across all plants studied. This metric provides insights into the consistency of diameter patterns among different plants and species. It is estimated as the linear relationship between the absolute values of the residuals from the DIDm model and the values predicted by it (Pagès, 2012).

Although not one of the five key parameters studied, the EL parameter is crucial for the ArchiSimple model. It represents the linear relationship between root diameter and root growth. Estimating EL directly impacts the overall architecture of the root system generated by ArchiSimple (Pagès, 2014). EL is calculated as the slope of the linear regression of growth rate against diameter.

5.6.1.2. Understanding the functions in the R script

Now that each parameter in the [script](#) has been explained, let's examine the various functions used to obtain them. It is important to note that the script provides a general method for obtaining the various parameters, without considering different varieties or treatments. As a result, the parameters will be given without discrimination, and statistical analyses will be necessary to determine if there are significant differences when multiple varieties or treatments are involved.

Outliers can introduce noise and obscure true trends in the data. Therefore, they are eliminated using the interquartile range (IQR), a measure of dispersion that describes the distribution of values in a data set (Vinutha, 2018). To calculate the IQR (Figure 20), the data set is divided into four equal parts, called quartiles. The IQR is the difference between the third quartile (Q3) and the first quartile (Q1), representing the range where the central values of the data set lie. This makes the IQR a robust measure of dispersion that is not influenced by extreme values. The script uses this method to eliminate outliers: values above $Q1 + 1.5IQR$ or below $Q3 - 1.5IQR$ are removed. For this purpose, the function 'diameter_outliers' was created (Degives, 2023), though it can be named differently depending on the nature of the data being processed.

```
diameter_outliers <- function(EXP1_new) {
  Q1 <- quantile(EXP1_new$diameter, 0.25)
  Q3 <- quantile(EXP1_new$diameter, 0.75)
  IQR <- Q3 - Q1
  lower <- Q1 - 1.5*IQR
  upper <- Q3 + 1.5*IQR
  data_clean <- EXP1_new %>% filter(diameter >= lower & diameter <= upper)
  return(data_clean)
}
```

Figure 20: Script of diameter_outliers.

To obtain the minimum diameter, the data is first grouped according to parent and parent name, and then the minimum value is found within each group. All this is done using the *aggregate* function. Once the data has been cleaned using the function created above, the average of the cleaned minimum values is calculated. For conversion purposes, the diameters had to be multiplied by 10.

The *get_minD* function (Figure 21) has therefore been created to allow users to take their data through the above stages quickly and easily.

```
get_minD <- function(yourdata) {
  # Aggregation of minimum diameter values by parent and parent_name
  Dmin <- aggregate(diameter ~ variety, data = yourdata , FUN = min)

  # Creation of the df Dmin_clean
  Dmin_clean <- data.frame()

  # Outlier filtering for all data
  cleaned_data <- diameter_outliers(Dmin)

  # Average of the minimum values cleaned
  mean_Dmin_clean <- mean(cleaned_data$diameter * 10, na.rm = TRUE)
  return(mean_Dmin_clean)
}
```

Figure 21: Script of get_minD.

For the maximum diameter (Figure 22), we start by filtering the data to retain only the nodal roots using the condition where the "root_ontology" variable is equal to "Root".

Using the max function on R, we can calculate the maximum diameter value by multiplying the largest diameter value in the cleaned data by 10 for conversion purposes.

All these operations are contained in the `get_maxD` function, which, like the previous function, is used to obtain the parameters more quickly. No discrimination has been made between the different varieties or treatments.

```
#MaxD

get_maxD <- function(yourdata) {
  # Retain only the nodal roots + filter outliers
  nodal_clean <- yourdata %>%
    filter(root_ontology == "Root") %>%
    diameter_outliers()

  # Display cleaned data
  print(nodal_clean)

  # Maximum diameter value multiplied by 10
  Dmax <- max(nodal_clean$diameter) * 10
  return(Dmax)
}
```

Figure 22: Script of `get_maxD`.

IBD (mean Inter-Branch Distance) is calculated as the mean value of the distance between neighbouring lateral branches. The data is first filtered to obtain the root branches and child branches (Figure 23). Their density is calculated using the formula $IBD = 1 / \text{child_density}$. The new numerical IBD variable (filtered from any NA data) is then added to the data. Finally, as no statistical analysis is carried out (yet again, it is up to the user to ensure this.), it is assumed that IBD for all varieties can be summarised as the mean of the IBD variable for all the data, which gives the mean of the distance between lateral branches. The `get_IBDmean` function is then created in the same way as the others presented above.

```
#IBD

get_IBDmean <- function(spelt.data) {
  # IBD column is added to spelt.data
  spelt.data <- spelt.data %>%
    mutate(IBD = ifelse(child_density != 0, 1 / child_density, NA))

  # data is filtered to include only numeric IBD values
  spelt.data <- spelt.data %>%
    filter(!is.na(IBD) & is.numeric(IBD))

  # we calculate the mean IBD for the entire dataset
  IBD_mean_all <- spelt.data %>%
    summarize(IBD_mean = mean(IBD, na.rm = TRUE))

  return(IBD_mean_all)
}
```

Figure 23: Script of `get_IBDmean`.

As a reminder, the DIDm (RDM in the model and in the script) is calculated from the slope of the linear regression between the diameters of the daughter roots and the mother root.

To make script writing less tedious, it is possible to create new columns (Figure 24) and add them to the existing ones. The ‘parent_diameter’ column is not a feature that can be extracted from Smartroot, but it is necessary for us. We initialise an empty data frame where we can store the new columns created and those selected. Using the pipe %>% operator, we can apply a series of operations. If the value of the parent column is not equal to ‘-1’ (which means that there is a parent), it retrieves the corresponding diameter of the parent root in the diameter column using the match function. If the value of the parent is equal to ‘-1’ (which means it has no parent), it assigns NA to the diameter_parent column. The ifelse function performs this conditional assignment.

```
new_spelt.data <- data.frame()

new_spelt.data <- spelt.data %>%
  select('root_name', 'root', 'diameter', 'root_ontology', 'parent', 'parent_name', 'child_density') %>%
  mutate(parent_diameter = ifelse(parent != '-1', diameter[match(parent, root)], NA))
```

Figure 24: The creation of a new column.

A linear regression model (mod) is fitted with the diameters of the child roots (diameter) as a function of the diameters of the parent roots (parent_diameter). Next, the *emmeans* function is used to calculate the estimated mean diameters of the daughter roots as a function of the fitted linear regression model. Finally, the function is used to calculate the estimated mean diameters of the daughter roots as a function of the fitted linear regression model. The *get_RDM* function contains all the operations just explained (Figure 25).

```
#RDM

get_RDM <- function(spelt.data) {
  # Fitting a linear regression model without interacting with the variety
  mod <- lm(diameter~ parent_diameter, data = spelt.data)

  # Calculation of estimated average diameters
  lsm <- emmeans(mod, ~ 1)

  # Return the summary of estimated averages
  return(summary(lsm))
}
```

Figure 25: Script of get_RDM.

As CVDD is defined as the linear relationship between the absolute value of the residuals of the DIDm model and the values estimated by it. The *lm* function is used to fit a linear regression model between the diameters of the daughter roots diameter and the diameter of the parent root parent_diameter (Figure 26). The predict function is used to calculate the predicted values from the fitted linear regression model. The residual function is used to calculate the residuals of the adjusted linear regression model.

```
#CVDD
get_CVDD <- function(spelt.data) {
  # Fitting a linear regression model
  mod <- lm(diameter ~ parent_diameter, data = spelt.data)

  # Calculate predicted values
  predicted_values <- predict(mod)

  # Calculate residuals
  residuals <- residuals(mod)

  # Fit a linear regression model between residuals and predicted values
  residuals_mod <- lm(abs(residuals) ~ predicted_values)

  # Calculate the average of the absolute values of the residuals divided by the predicted values
  mean_abs_residuals <- mean(abs(residuals) / predicted_values)

  # Return coefficient of variation CVDD
  return(mean_abs_residuals)
}
```

Figure 26: Script of get_CVDD.

The function created, *get_EL* (Figure 27), returns the slope of the relationship between growth and diameter, which is estimated from a linear regression model fitted to the data. The aim is to understand the relationship between growth and diameter.

```
get_EL <- function(primary) {
  # Fit a linear model with growth and diameter

  mod <- lm(growth ~ diameter, data = spelt.data)

  # Obtain the model slope coefficient
  slope <- coef(mod)

  # Return the slope of the relationship
  return(slope)
}
```

Figure 27: Script of get_EL.

5.6.1.3. Running the script

Now that the functions have all been created, we'll simply run it on a sorghum dataset (Degives,2023). The data includes information on six varieties: Amiggo, Hyperion, Biggben, Vegga and Juno. As this section is only concerned with verifying the script, and as it does not discriminate between sorghum varieties, they will not be detailed in this section.

If the study was carried out on several species, whose measurements were recorded in several different excels, this script combines the data from several CSV files into a single dataset by adding a column indicating the variety to which each row belongs. The loop then retrieves all the CSV files for the different species and combines them using the *rbind* function (Figure 28).

```
#this script combines the data from several CSV files into a single dataset
#adding a column indicating the variety to which each row belongs.
for (variete in c("Amiggo","Biggben","Hyperion","Juno","Swingg","Vegga")) {
  paste0("C:/Users/mkerd/OneDrive/Documents/MEMOIREDATA/End_growth_root_data/0.global_root_data/",variete,".csv") %>%
  read.csv() %>%
  mutate(variety = factor(variete)) %>%
  rbind(end.global,.) -> end.global
}
```

Figure 28: Combining different CSV files.

To use the R script containing the various functions described above, the *source()* function is simply used. In the master thesis (Degives,2023) in which I am using the data, some parameters have been obtained from the data at the beginning, while others are based on the data at the end. The same approach was used to test the script containing the functions. Thus, all the functions were used to find the main parameters. When the function script is loaded via the *source()* function, the list of functions is displayed in the environment section at top right, as shown in figure 29.

Functions		
diameter_outliers	function (data)	
get_CVDD	function (lateral.clean)	
get_EL	function (primary)	
get_IBDmean	function (early.data)	
get_maxD	function (end.data)	
get_minD	function (end.data)	
get_RDM	function (lateral.clean)	

Figure 29: Functions displayed in the environment section.

Since my master's thesis is a syllabus designed to help users use an R script, it is more advantageous to include the values and results of the R script directly in the main text. So, to illustrate the analysis process of our sorghum data, we'll walk through several R script commands and their outputs, explaining each step in detail. Any interpretation will be made in the discussion section.

First, we calculate the minimum diameter of the sorghum roots using the *get_minD* function (Figure 30). This output indicates that the smallest root diameter in our dataset is approximately 0.153 mm.

```
> minD <- get_minD(end.data)
> print(minD)
[1] 0.1533811
```

Figure 30: Minimum Diameter Calculation.

Next, we determine the maximum diameter and extract the associated data using the *get_maxD* function (Figure 31) and it shows that the largest root diameter is approximately 6.985 mm.

```
> maxD <- get_maxD(end.data)
[1] root_name      root          diameter      root_ontology  parent
[6] parent_name     variety       child_density parent_diameter ratio_diameter
<0 lignes> (ou 'row.names' de longueur nulle)
> print(maxD)
[1] 6.984537
```

Figure 31: Maximum Diameter Calculation.

We then calculate the mean inter-branch distance (IBD) using early growth data (Figure 32) (This was the author's choice, as the roots are easier to see at the beginning of development than at the end.). The mean IBD of about 1.6760 mm is, as a reminder, the average spacing between branches in the early growth stage.

```
> IBD <- get_IBDmean(early.data)
> print(IBD)
IBD_mean
1 1.676096
```

Figure 32: IBD Diameter Calculation.

As the RDM represents the slope of the linear regression of the diameter of lateral roots versus their mother root. It is therefore necessary to process the data in order to keep only what is of interest to us. A script (Figure 33) is used to filter, scale and clean the data before storing it in a new data frame. The process begins by initializing an empty data frame called *lateral.clean*. This data frame will subsequently contain the filtered and cleaned data. The script then loops

through each sorghum variety specified: Amiggo, Biggben, Hyperion, Juno, Swingg and Vegga. For each variety, it filters the end.data dataset to retain only those rows where the variety matches the current variety in the loop and where the root ontology is “Lateral Root”. This ensures that only lateral root data relevant to each variety is selected.

```
#Filter the data to retain only the lateral roots of the varieties.
lateral.clean <- data.frame()
for (variete in c("Amiggo","Biggben","Hyperion","Juno","Swingg","Vegga")) {
  filter(end.data, variety==variete,root_ontology==' Lateral root') %>%
    mutate(diameter=diameter*10) %>%
    mutate(parent_diameter=parent_diameter*10) %>%
    diameter_outliers(.) %>%
    rbind(lateral.clean,.) -> lateral.clean
}
```

Figure 33: Script to obtain lateral.clean.

The figure 34 provides us the estimated RDM which is 0.19.

```
> RDM <- get_RDM(lateral.clean)
> print(RDM)
  1      emmean      SE    df lower.CL upper.CL
overall  0.19 0.000855 7462   0.188   0.191

Confidence level used: 0.95
```

Figure 34: Script to obtain RDM.

Then, to assess the variability, we calculate and obtain CVDD of approximately 0.255 (Figure 35).

```
>
> CVDD <- get_CVDD(lateral.clean)
> print(CVDD)
[1] 0.2550709
```

Figure 35: Script to obtain CVDD.

And finally, we obtain our sixth parameter in the same way as the others (Figure 36).

```
> EL <-get_EL(primary)
> print(EL)
(Intercept)      diameter
-0.7258118    33.0344257
```

Figure 36: Script to obtain EL.

5.7. Checking the script effectiveness

In the preceding section, we verified the functionality of the script by ensuring that each time a parameter was requested, the code provided a corresponding response. This initial step allowed us to confirm the operational integrity of the script. However, it was imperative to ascertain the accuracy of the output values and evaluate the script's capability to generate expected results. To achieve this, we utilized various datasets, some of which had been previously employed in master's theses. This approach facilitated direct comparisons with known values, ensuring the reliability of our analysis.

The first dataset comprised information on sorghum, a cereal grain renowned as broomcorn or milo, serving as a vital food source in many regions worldwide. Sorghum's resilience in hot and arid conditions can be attributed, in part, to its robust root system. Initially characterized by a single main root, sorghum's root architecture transitions into a network of roots emanating from the stem. These roots exhibit extensive lateral spread and penetration depths, reaching at least 1.5 meters into the soil, enabling efficient water and nutrient uptake (Hostetler et al., 2024).

The second dataset included data on wheat and spelt, two cereals sharing similar characteristics to sorghum.

Lastly, the third dataset encompassed spinach, butternut, and pumpkin.

5.7.1. Sorghum and its many varieties

The comparison between both studies, utilizing identical datasets (Degives, 2023), serves to affirm the efficacy of the script. Through this comparative analysis, the functionality of the script is validated. During the course of analysis, Nicolas observed that, with the exception of the Vegga variety, it was difficult to distinguish the other species. Consequently, it was observed that the average minimum diameter across all varieties, excluding Vegga, matched 0.1574 (Figure 37). Our analysis presented a slightly lower minimum mean diameter of 0.1534, as generated by the script.

* **lsmeans**:

variety	lsmean	SE	df	lower.CL	upper.CL
Amiggo	0.1581	0.009185	389	0.1401	0.1762
Biggben	0.1388	0.009921	389	0.1193	0.1583
Hyperion	0.1777	0.008995	389	0.16	0.1954
Juno	0.1585	0.009252	389	0.1403	0.1767
Swingg	0.1502	0.00946	389	0.1316	0.1688
Vegga	0.1297	0.01018	389	0.1097	0.1497

Figure 37: Estimated Least Square Means and 95% Confidence Intervals for Different Varieties.

It was also imperative for Nicolas to assess similarities or disparities by examining the distributions and using an ANOVA model to discern significant differences. In addition, the aim was to identify the largest diameters, with Dmax representing the maximum diameter observed in the nodal root distribution. Consequently, the conclusion drawn was: “The overall Dmax for all six varieties is 6.9845”. It is noteworthy that the script gave exactly the same result (cf “Running the script” section).

A similar scenario occurred regarding the Inter-Branch Distance (IBD) analysis, in which the conclusion was: “Average IBD for all six varieties = 1.6760”. Here again, our script produced an identical result.

As for the slope of the linear regression of the diameter of the lateral roots vs. their mother root, the graph generated by Nicolas' script made it possible to observe the relationship between the diameter of the parent roots and that of the daughter roots, highlighting the differences between

the varieties (Figure 38). It is therefore not possible to conclude that the six varieties have the same slope and can be summed up in the same RDM value, but in the script of functions created from scratch this is the case and the value found is 0.19, which seems to be an average for all the varieties (Figure 39).

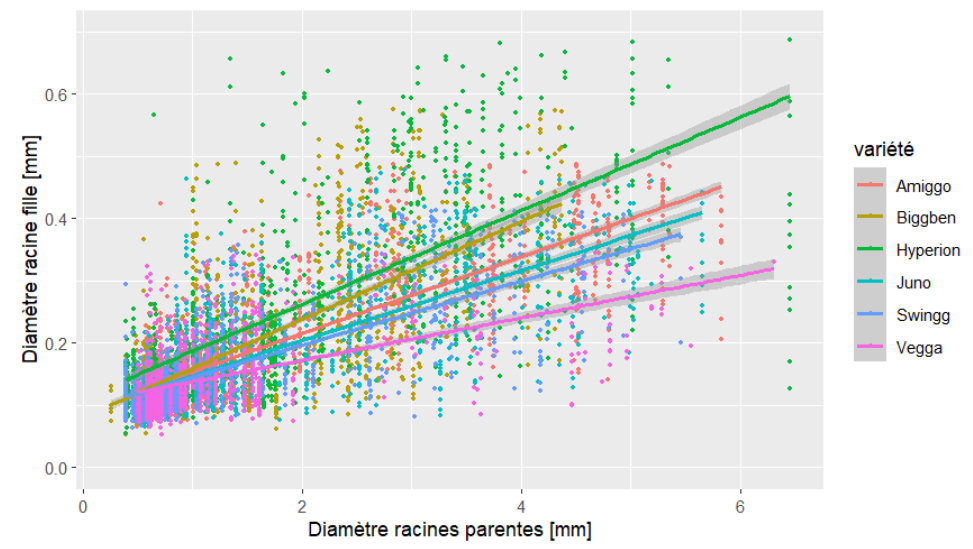


Figure 38: Slope of the linear regression of the diameter of the lateral roots vs. their mother root (Degives,2023).

variety	emmean	SE	df	lower.CL	upper.CL
Amiggo	0.1874	0.001716	7452	0.184	0.1908
Biggben	0.2022	0.002043	7452	0.1981	0.2062
Hyperion	0.228	0.00223	7452	0.2236	0.2324
Juno	0.1779	0.001907	7452	0.1742	0.1817
Swingg	0.173	0.00204	7452	0.169	0.177
Vegga	0.1567	0.002259	7452	0.1523	0.1611

Figure 39: RDM of all six varieties (Degives,2023).

CVDD concluded that "The average of the |residues|/fitted.values 0.2448" and the function script allows us to obtain the same value.

Finally, we obtain the same value for EL as in the previous work (Figure 40). This test shows us that the functions work correctly and that the information given is what we are looking for, but it is important to add that without statistical analysis, if the varieties or treatments are different, it is not relevant to take the average of the values, as there could be a bias.

	Estimate	Std. Error	t value	Pr(> t)
** (Intercept) **	-0.7258	0.9639	-0.753	0.4624
diameter	33.03	16.95	1.949	0.06904

Observations	Residual Std. Error	R^2	Adjusted R^2
18	0.4806	0.1919	0.1414

Figure 40: EL value for the six varieties (Degives,2023).

5.7.2. Wheat and spelt analysis

Analyzing wheat and spelt datasets from another project presented an opportunity to further test the script's functionality. The datasets comprised values from different experimental manipulations for each species. Since the script doesn't differentiate between species or treatments, I combined the wheat files and introduced an 'experiment' column, converting it into a factor for easier data manipulation (Figure 41). This approach facilitates swift and efficient statistical analyses based on experimental conditions. However, for simplicity, I focused solely on extracting the relevant parameters of interest from the datasets.

```
# spelt.global dataframe initialization
spelt.global <- data.frame()

# Loop for each experience
for (exp in c("diameter_spelt1", "diameter_spelt2")) {
  file_name <- paste0("C:/Users/mkerd/OneDrive/Documents/MEMOIREDATA/EpautreBlé/", exp, ".csv")
  data <- read.csv(file_name)

  # Add "experiment" column with current experiment value
  data$experiment <- exp

  # Merge with global dataframe
  spelt.global <- rbind(spelt.global, data)
}

# Convert "experiment" column to factor
spelt.global$experiment <- factor(spelt.global$experiment)
```

Figure 41: An example of data restructuring.

The process and way of acquiring the data have not changed from the section above, but it would be relevant to add the way in which the CSV files containing the “growth” metric, named

ER_E1 and ER_E2 for spelt and ER_B1 and ER_B2 for wheat respectively, have been merged. The merge created is then itself merged with the previous merge (Figure 42).

In this way, we have the diameter and growth metrics in the same file, and the acquisition of the ArchiSimple EL parameter is made possible.

```
ER_Spelt <- rbind(ER_E1, ER_E2)
ER_Wheat <- rbind(ER_B1, ER_B2)

ER_Spelt$growth <- factor(ER_Spelt$growth)
ER_Wheat$growth <- factor(ER_Wheat$growth)

# Merge ER_Spelt with spelt.global
merged_spelt_data <- merge(spelt.global, ER_Spelt, by = "root_name", all.x = TRUE)

# Display the first few rows of the merged dataframe
head(merged_spelt_data)
```

Figure 42: Merge all CSVs.

The script was initially executed for spelt values exclusively. Analysis of the spelt dataset revealed a range of root diameters, with the minimum and maximum diameters observed approximately at 0.21 mm and 0.52 mm, respectively. Subsequently, the mean inter-branch distance (IBD) was computed to be approximately 1.64, while the RDM was determined to be 0.271. Furthermore, the coefficient of variation of diameter distribution (CVDD) was calculated to be around 0.174. Finally, the script provided an EL value of 119.82 for spelt.

For wheat, the analysis yielded a minimum diameter of 0.203 mm and a maximum diameter of 0.502 mm. The mean inter-branch distance (IBD) was determined to be 1.81, with an RDM value of 0.271 and a CVDD of 0.174. Additionally, an EL value of 102.56 was obtained for wheat.

While some parameters may pose challenges for verification, it is essential to note that the minimum and maximum diameters for both cereals were observed to be 0.203-0.21 mm and 0.502-0.52 mm, respectively. Referring to an article by Yamaguchi (2002) on root diameter distribution in various crops provides valuable insights for interpreting these results. The article reports an average diameter for wheat falling within the range of 0.18-0.20 mm, which aligns closely with the minimum diameters observed for both wheat and spelt. Furthermore, the article underscores a wider range of diameters for roots exceeding 0.20 mm, consistent with our maximum diameter measurements.

5.7.3. Spinach, butternut and pumpkin analysis

The script underwent a third round of testing using three distinct CSV files containing SmartRoot outputs for spinach, pumpkin, and butternut, as detailed in Heinrich-Thuet's thesis (2023). Each dataset was processed through the same script, enabling extraction of all key parameters of the model except EL. This omission was due to insufficient data in the CSV files, which lacked growth-related information such as position or date—suggesting that the data did not originate from a rhizotron plot. Once again, the script was configured to select specific columns—"root_name," "root," "diameter," "root_ontology," "parent," "parent_name," and "child_density"—from each dataset. Subsequently, a dataframe was created incorporating these columns, with the addition of a new "parent_diameter" column specifically to the butternut dataset (Figure 43).

Data		
▶ Butternut	851 obs. of 21 variables	
▶ butternut.data	851 obs. of 9 variables	
▶ pumpkin	1909 obs. of 21 variables	
▶ pumpkin.data	1909 obs. of 9 variables	
▶ Spinach	372 obs. of 21 variables	
▶ Spinach.data	372 obs. of 9 variables	

Figure 43: Environment containing the new dataframes.

When analyzing the butternut root dataset, the extracted metrics allowed us to conclude that the minimum root diameter was around 0.294 mm, while the maximum diameter reached around 1.163 mm. The average inter-branch distance (IBD) was calculated to be around 8.00 mm. RDM has a slope of 0.433. The coefficient of variation of diameter distribution (CVDD) was determined to be approximately 0.197.

Naturally, the same analysis was carried out on spinach and revealed the minimum root diameter, extracted using the *get_minD* function, to be around 0.321 mm. Conversely, the maximum diameter, calculated using the *get_maxD* function, was significantly higher, measuring around 0.989 mm.

Next, the script output IBD, which was calculated at around 26,917 mm using the *get_IBDmean* function, the RDM slope of 0.404. In addition, CVDD was determined to be around 0.16.

Finally, the script was run one last time on the pumpkin. When analyzing the pumpkin root dataset, the extracted metrics allowed us to conclude that the minimum root diameter was around 0.261 mm, while the maximum diameter reached around 1.622 mm. Average IBD was calculated to be around 7.252 mm. RDM resulted in an overall mean of 0.454 mm. The coefficient of variation (CVDD) was determined to be approximately 0.253.

It's noteworthy to compare these findings with root diameter measurements reported in the previous master's thesis (Heinrich Thuet, 2023).

A range of minimum diameters from 0.282 mm to 0.399 mm was observed, which aligns well with the minimum diameters observed in our study for all three plants. Additionally, the previous study reported maximum diameters ranging from 0.433 mm to 0.747 mm but the script gave higher values.

As for IBD, the previous work observed an oscillation between 4.89 mm and 66.76 mm for spinach samples. For butternut samples, between 4.19 mm and 10.19 mm, and for pumpkin samples, between 3.77 mm and 8.9 mm. Everything seems to fit within the ranges given by Nicolas.

5.8. Analysis of Root Morphological Traits Across Diverse Plant Genotypes

The remainder of this section was designed to analyze the morphological characteristics of the roots of the various plant species seen separately and their respective genotypes. Specifically, we examined the minimum diameter (Dmin), maximum diameter (Dmax), the inter branch distance (IBD), the estimate of the relationship between the diameters of the daughter and parent roots (RDM) and coefficient of variation (CVDD).

5.8.1. Plants and Varieties Analyzed

- Spelt: genotypes included R12, R130, R15, R208, R238, R23, R43, and R47.
- Wheat: genotypes included D, GV, HII, HIV, J, O, and T.
- Sorghum, varieties included Amiggo, Biggben, Vegga, Juno, Hyperion, and Swingg.
- Pumpkin, Spinach, and Butternut: each plant was represented by a single variety: Pumpkin, Spinach, and Butternut.

5.8.2. Minimum diameter

The analysis of Dmin values across various plant genotypes, as described in the preceding section, revealed noteworthy disparities (Figure 44). Among sorghum varieties, Vegga exhibited the smallest Dmin at 0.144 mm, while other sorghum cultivars such as Biggben (0.150 mm), Swingg (0.154 mm), Amiggo (0.163 mm), and Juno (0.163 mm) showcased slightly larger but still fine root structures. This consistent trend among sorghum variants underscores their inherent genetic inclination towards developing slender roots, a trait advantageous for thriving in arid climates (Frontiers, 2023).

Spinach registered a Dmin of 0.321 mm, positioning it among varieties with thicker roots compared to sorghum. Butternut presented a Dmin of 0.315 mm, similar to spinach. Pumpkin, with a Dmin of 0.300 mm, also fell into this category.

Spelt varieties exhibited moderate Dmin values, with R12 (0.223 mm) and R47 (0.231 mm) at the higher end of the spectrum, and R130 (0.200 mm) and R208 (0.210 mm) at the lower end.

Wheat varieties showcased Dmin values within a narrow range, with HII (0.214 mm) and HIV (0.217 mm) exhibiting the finest roots, while J (0.191 mm) displayed the least fineness.

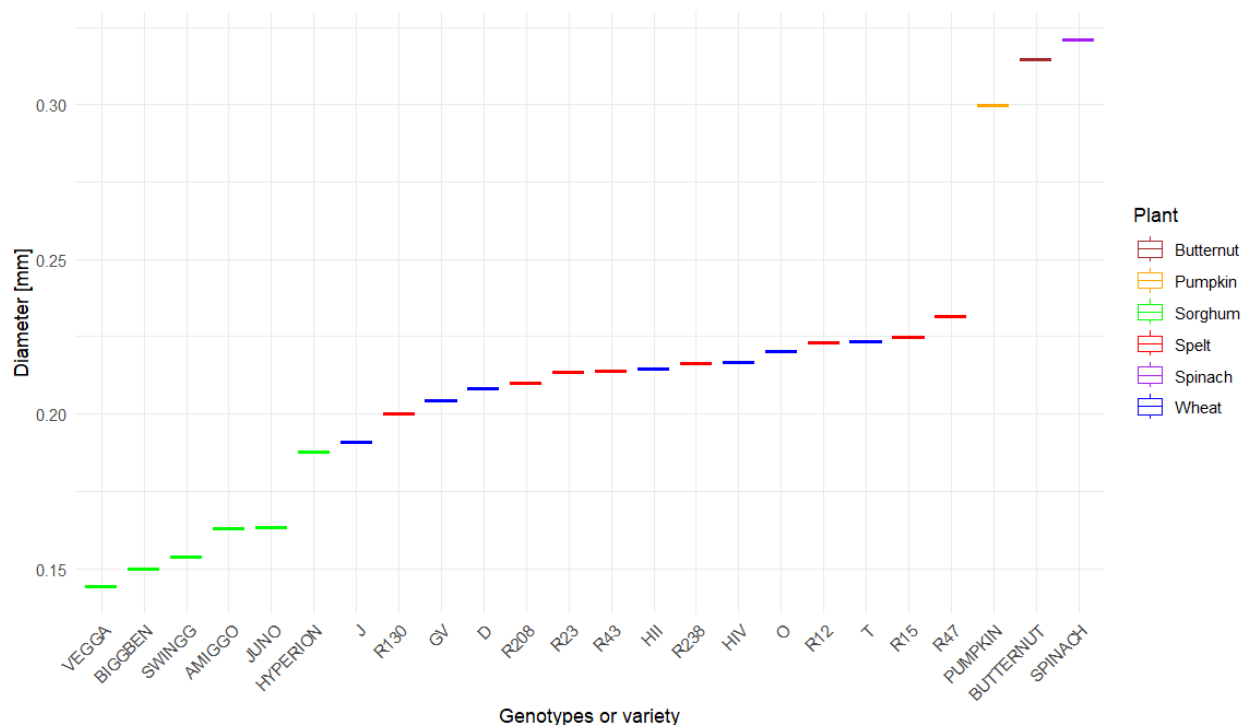


Figure 44: Distribution of the finest root diameter according to the genotype or variety.

5.8.3. Maximum diameter

Sorghum genotypes, namely Amiggo, Biggben, Hyperion, Juno, Swingg, and Vegga, exhibit relatively large maximum diameters, ranging from approximately 4.37 to 6.98 (Figure 45).

In contrast, wheat genotypes such as D, GV, HII, HIV, J, O, and T display notably smaller maximum diameters, ranging from around 0.45 to 0.54.

Furthermore, other species, including butternut, pumpkin, spelt, and spinach, each present unique maximum diameter ranges distinct from both sorghum and wheat. For instance, butternut and pumpkin showcase maximum diameters around 1.32 and 1.62, respectively, while spelt and spinach demonstrate values ranging from approximately 0.46 to 0.99.

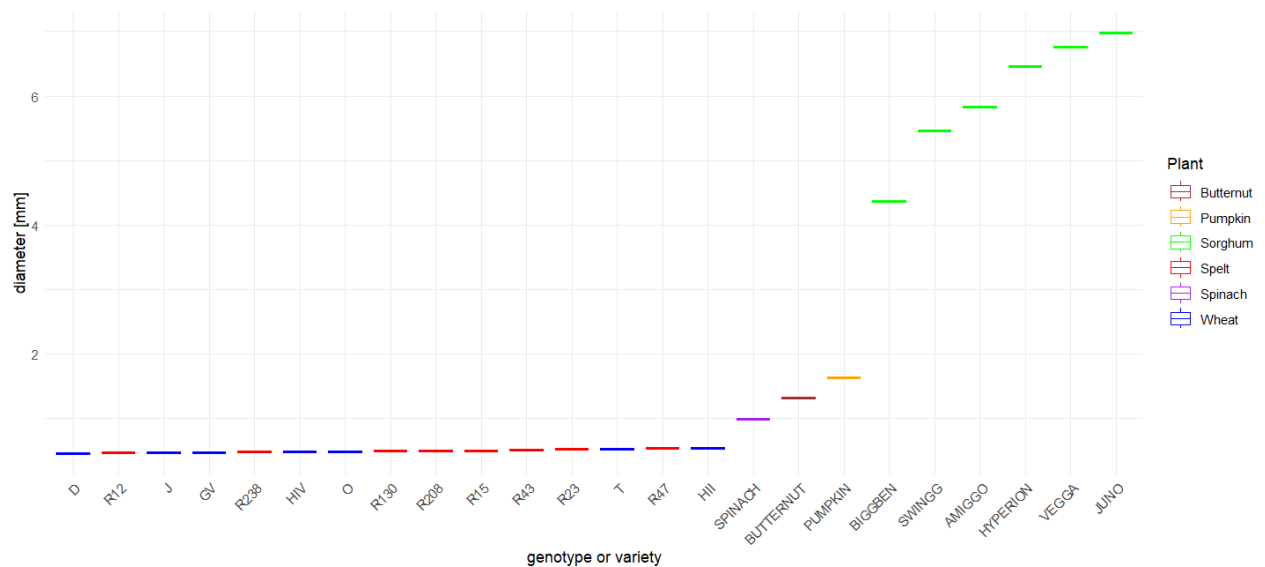


Figure 45: Distribution of the maximum root diameter according to the genotype or variety.

5.8.4. Inter-branch distance (IBD)

Wheat genotypes, represented by D, GV, HII, HIV, J, O, and T, display a broader range of inter-branch distances, with values ranging from around 15.69 to 24.02 (Figure 46).

In contrast, sorghum genotypes such as Amiggo, Biggben, Hyperion, Juno, and Vegga exhibit relatively low inter-branch distances, ranging from approximately 1.59 to 1.87. This suggests a common trait pattern within Sorghum species.

On the other hand, butternut, pumpkin, spelt, and spinach each present distinct inter-branch distances compared to both sorghum and wheat. For instance, butternut and pumpkin showcase relatively high inter-branch distances around 8.00 and 7.25, respectively, while spelt and spinach demonstrate values ranging from approximately 14.10 to 26.92.

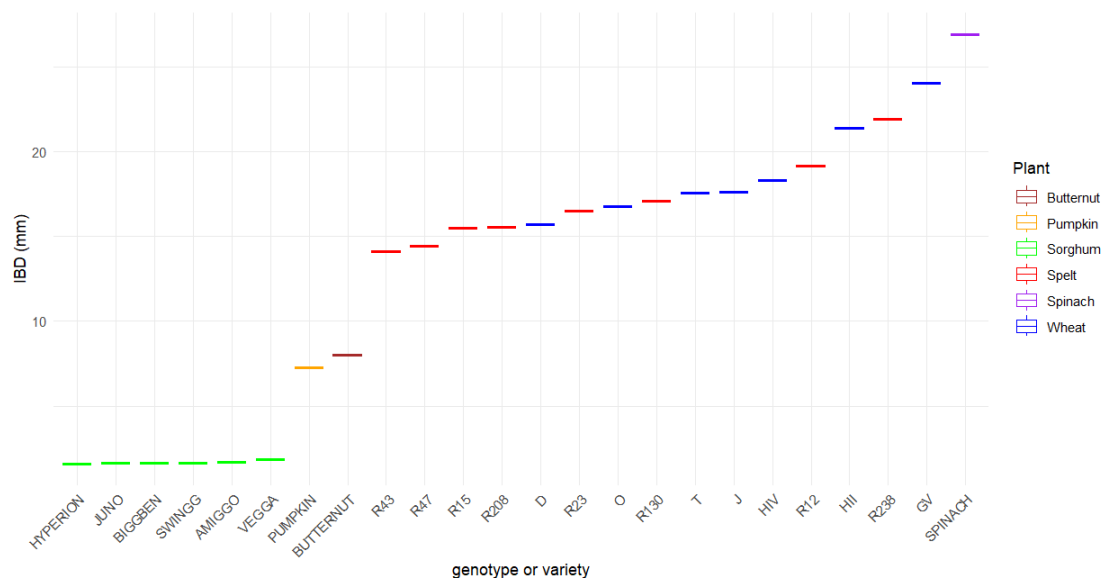


Figure 46: Distribution of IBD according to the genotype or variety.

5.8.5. The slope of the linear relationship between the diameter of a lateral root and that of its parent root (RDM)

The RDM values of sorghum genotypes, including Amiggo, Biggben, Hyperion, Juno, Swingg, and Vegga, are relatively low, ranging from 0.165 to 0.22 (Figure 47).

In contrast, wheat genotypes represented by D, GV, HII, HIV, J, O, and T, exhibit a broader range of RDM values, ranging from approximately 0.305 to 0.343.

On the other hand, other species such as butternut, pumpkin, spelt, and spinach again demonstrate differences from both sorghum and wheat. For example, butternut and pumpkin show relatively higher RDM values of around 0.456 and 0.457, respectively. Spinach displays a RDM value of approximately 0.447.

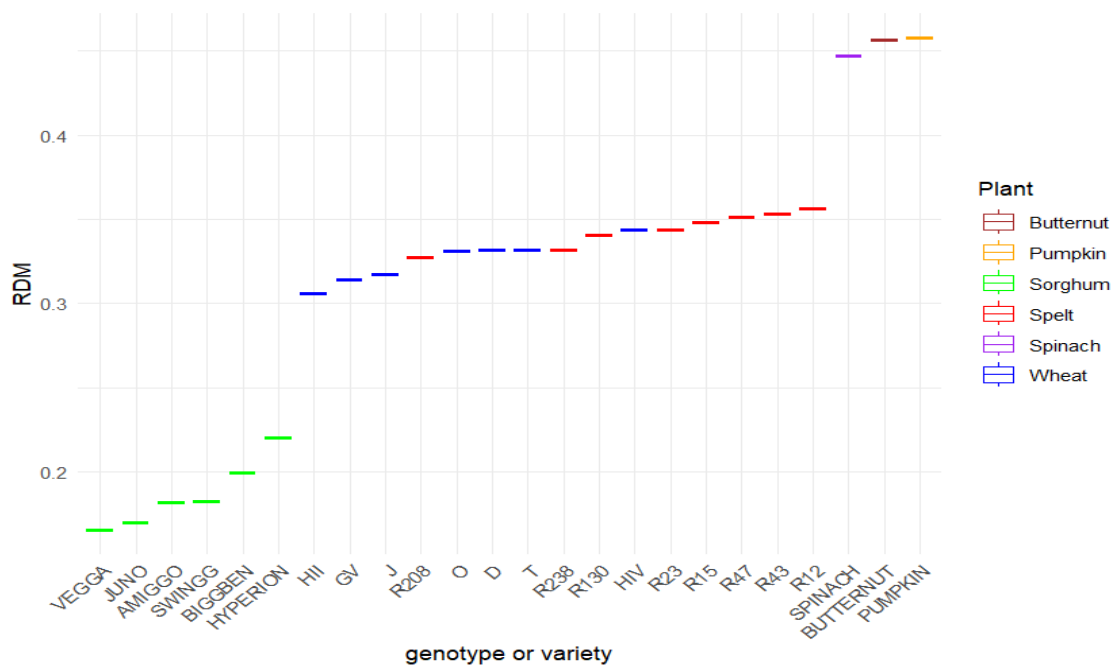


Figure 47: Distribution of RDM according to the genotype or variety.

5.8.6. The coefficient of variation (CVDD)

Despite belonging to different plant species, butternut, spinach, spelt and wheat show CVDD values that overlap with those observed in certain sorghum genotypes (Figure 48).

Spelt varieties (R12, R130, R15, R208, R238, R23, R43, and R47) exhibit CVDD values, ranging from approximately 0.152 to 0.198. Similarly, wheat varieties (D, GV, HII, HIV, J, O, and T) demonstrate CVDD values ranging from about 0.162 to 0.218.

Amiggo, Biggben, Hyperion, Juno, Swingg, and Vegga, representing sorghum genotypes, exhibit a wide range of CVDD values. These values range from approximately 0.160 to 0.326.

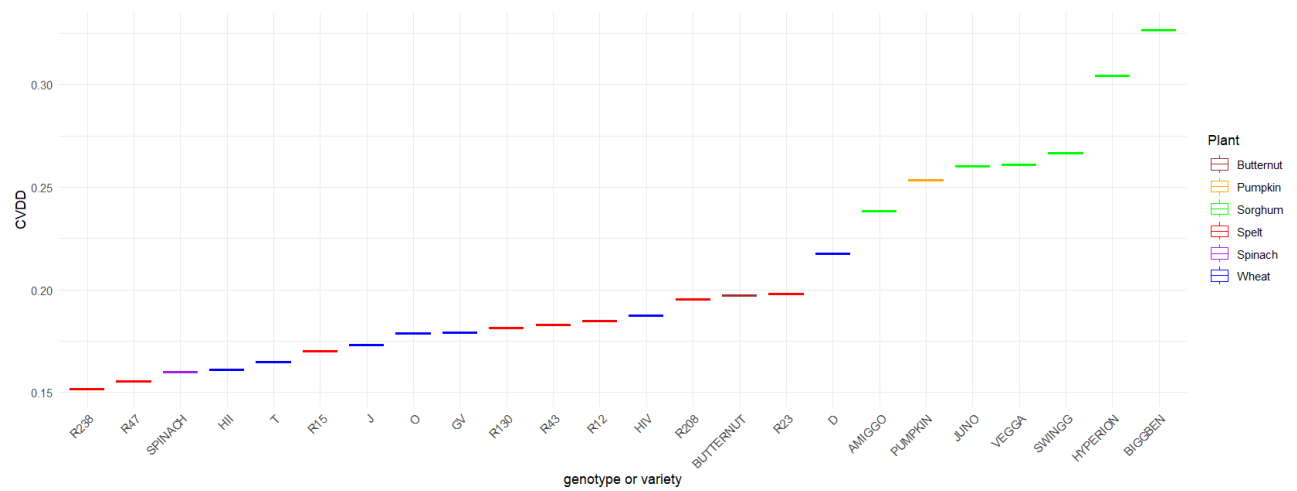


Figure 48: Distribution of CVDD according to the genotype or variety.

6. General discussion

Up to now, it has always been possible to test the script and compare certain parameters with existing work. This does not mean, however, that the values found correspond perfectly to reality. Indeed, it's complicated to give a full critique of the values and what they should have been, as my work has focused on creating a guideline for the user. Nonetheless, it's worth using common sense when acquiring results. There were inconsistencies in the data. Incomplete datasets for some plants (spinach, butternut, and pumpkin) probably led to differences between calculated averages and existing data. Additionally, the ways of calculating minimum and maximum root diameters (D_{min} and D_{max}) differed. The script's method contrasted with Nicolas' approach, which relied on specific percentiles (10th and 90th).

Another issue was missing harvest timing information for wheat and spelt. This could affect estimates of how fast their roots were growing. These inconsistencies highlight the importance of standardized procedures and data collection methods for accurate comparisons of root traits between different plant types and genetic variations.

Looking at specific plant groups, sorghum, known for its genetic adaptation to dry environments (Frontiers in Plant Science, 2023), had smaller minimum diameters (D_{min}) as expected, compared to other plants. Wheat and spelt, being in the same family, unsurprisingly had similar D_{min} ranges. Pumpkin, a completely different species from sorghum and wheat, showed values within the normal range (Guo et al., 2023).

However, there was a surprising difference in the maximum diameter (D_{max}) values between sorghum and wheat. This prompted further investigation, revealing the same differences in the raw data (CSV format). Possible reasons for this could be variations in how the data was collected, or maybe when the plants were harvested. The unknown harvest time for wheat and spelt might explain the high values calculated for their EL, suggesting an altered relationship between root diameter and growth.

Overall, analyzing various root characteristics, including minimum diameter (D_{min}), maximum diameter (D_{max}), spacing between root branches (inter-branch distance), the ratio of side root thickness to main root thickness (root diameter ratio), and the variation in root thickness across the plant (The coefficients of variation for daughter diameters (CVDD)), across different variations within the same plant species strengthens the idea that these traits

are important for understanding how root systems differ. By examining these traits, we were able to confirm again some important factors that influence root architecture across different plant types and genetic variations.

There's an important point, though, regarding the script. This re-analysis showed that we need to be careful about how we interpret the values it gives us. Simply averaging values within a plant group without doing proper statistical analysis can be misleading. A more reliable approach involves statistical tests (like calculating a p-value) to draw accurate conclusions. The script is a time-saving tool, but users need to use their judgment based on their specific data and what they're trying to learn. If you're analyzing scans from the same plant, with only one genetic variation and under identical conditions, the script is enough to get the six root architecture parameters. But for broader comparisons or studies involving different plant variations or methods, a more thorough statistical approach is necessary.

6.1. Rhizovision: could it potentially replace Smartroot?

From the start, another aim of this master's thesis was to evaluate whether RhizoVision could potentially replace SmartRoot. Given RhizoVision's fully automated nature, it was hypothesized that it might offer advantages in terms of ease of use and overall speed. However, on closer examination, it became clear that each tool has distinct strengths and limitations.

RhizoVision is effective for comprehensive measurements of root morphology and geometry. The software's ability to calculate parameters such as width-depth ratio, network area, and perimeter enhances our visualization of root structure. However, RhizoVision does not provide detailed information on individual root characteristics, such as child density, insertion angles, and distances between the base and apex. Furthermore, while RhizoVision can provide the maximum diameter without further processing in R Studio, access to the raw data is needed to obtain the minimum diameter. This raises questions about whether it truly offers a time-saving advantage.

On the other hand, SmartRoot excels in analyzing the characteristics and development of individual roots. Its CSV output is easier to manipulate to derive the parameters needed for the model. However, SmartRoot lacks the comprehensive measurements of root morphology and geometry that RhizoVision offers. It does not provide measurements such as network surface,

convex surface, and solidity, which limits its ability to fully characterize root system architecture.

In conclusion, while RhizoVision and SmartRoot each offer unique advantages, RhizoVision alone is not a complete solution for acquiring the parameters required for the ArchiSimple model. However, users can benefit from using both tools together, drawing on the strengths of each to gain a comprehensive understanding of root structure, morphology, and development.

7. Conclusion

Root system architecture is crucial for plant growth, productivity, and environmental stress resistance. However, studying root systems poses challenges due to their development in soil and their delicate nature. Software has been developed to enhance data acquisition and visualization, along with computer models for scenario analysis. These tools necessitate precise root scanning, which depends on optimal growth conditions. Thus, this research focuses on improving data acquisition before modeling. A set of protocols has been established to facilitate experimental procedures, minimizing the need for extensive information searches.

Following the presentation of these protocols, the research also introduces root analysis software: the semi-automated SmartRoot and the fully automated RhizoVision. These tools generate files containing data such as root diameters, ontology, and lateral density, which can be further analyzed using models like ArchiSimple.

ArchiSimple is a C++ model designed to study root growth dynamics and structural properties across various plant species and environmental conditions. It offers a three-dimensional representation of root system development over time, emphasizing fundamental growth processes. ArchiSimple requires 24 parameters for accurate simulation, and an R script was created to obtain five critical parameters (D_{min} , D_{max} , $CVDD$, $DIDm$, and IBD) that capture the root system's characteristics effectively.

The R script was initially tested with CSV data from SmartRoot outputs, including six Sorghum varieties, wheat, spelt, spinach, and two cucurbits. The script's outputs were compared with existing data from two master's theses and scientific literature, confirming that it produced consistent and plausible values. This analysis reaffirmed the significance of the five parameters in influencing root architecture across species and genotypes. However, it also emphasized the necessity of detailed statistical analysis, including p-values, to ensure accurate conclusions. While the script saves time, it must be used correctly and adapted to specific data and analysis purposes.

The potential of replacing SmartRoot with the fully automated RhizoVision was also considered. RhizoVision provides a comprehensive overview of root morphology and geometry but lacks detailed individual root characteristics essential for the ArchiSimple model.

Consequently, combining SmartRoot and RhizoVision offers a more complete understanding of root structure, morphology, and development.

Although the research achieved its overall objective, there is still room for improvement. The protocols could not be fully implemented despite being surveyed at the experimental site. Moreover, although five parameters were identified as crucial, ArchiSimple still requires 24 parameters. Future work could involve integrating the missing parameters into the script and collecting theoretical data for each species, further aiding users.

Bibliographical references

- Australian Society of Plant Scientists. (2018). Root system architecture. Retrieved April 2, 2024, from <https://www.rseco.org/content/412-root-system-architecture.html>
- ChéFaire. (2023, February 23). *Root phenotyping: measuring roots with ImageJ SmartRoot plugin* [Video]. ChéFaire.
<https://www.youtube.com/watch?v=UDi1AdCzR5k>
- Cambridge Dictionary. (n.d.). Protocol. In Cambridge Dictionary. Retrieved March 10, 2024, from <https://dictionary.cambridge.org/dictionary/english/protocol>
- Degives, N. (2023). Quantification et modélisation du système racinaire de sorgho.
<https://hdl.handle.net/2078.1/thesis:40953>
- Degives, N. (2023). LBIRA2200-memoire. GitHub.
<https://github.com/ndegives/LBIRA2200-memoire>
- Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., Klimešová, J., Zadworny, M., Poorter, H., Postma, J. A., Adams, T. S., Bagniewska-Zadworna, A., Bengough, A. G., Blancaflor, E. B., Brunner, I., Cornelissen, J. H. C., Garnier, E., Gessler, A., Hobbie, S. E., Meier, I. C., Mommer, L., Picon-Cochard, C., Rose, L., Ryser, P., Scherer-Lorenzen, M., Soudzilovskaia, N. A., Stokes, A., Sun, T., Valverde-Barrantes, O. J., Weemstra, M., Weigelt, A., Wurzbürger, N., York, L. M., Batterman, S. A., Gomes de Moraes, M., Janeček, Š., Lambers, H., Salmon, V., Tharayil, N., & McCormack, M. L. (2021). A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytologist*, 232(3), 973-1122.
<https://doi.org/10.1111/nph.17572>

- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., Bardgett, R. D., De Deyn, G. B., Johnson, D., Klimešová, J., Lukac, M., McCormack, M. L., Meier, I. C., Pagès, L., Poorter, H., Prieto, I., Wurzbürger, N., Zadworny, M., Bagniewska-Zadworna, A., Blancaflor, E. B., Brunner, I., Gessler, A., Hobbie, S. E., Iversen, C. M., Mommer, L., Picon-Cochard, C., Postma, J. A., Rose, L., Ryser, P., Scherer-Lorenzen, M., Soudzilovskaia, N. A., Sun, T., Valverde-Barrantes, O. J., Weigelt, A., York, L. M., & Stokes, A. (2021). Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytologist*, 232(3), 1123-1158. <https://doi.org/10.1111/nph.17072>
- Guide de l'utilisateur Perfection V800 Photo/V850 Pro. (n.d.). Figure on page 70. Retrieved June 2, 2024, from <https://files.support.epson.com/docid/cpd4/cpd41531.pdf>
- Guo, H., Yang, H., Guo, W., Li, X., & Chen, B. (2023). Defense response of pumpkin rootstock to cadmium. *Scientia Horticulturae*, 308, 111548. <https://doi.org/10.1016/j.scienta.2022.111548>
- Heinrich-Thuet, N. (2023). Étude du développement racinaire de cultures maraichères post-conversion de prairie selon différentes modalités de culture. Approche via modélisation. <https://dial.uclouvain.be/memoire/ucl/object/thesis:40548>
- Hostetler, A. N., Morais de Sousa Tinoco, S., & Sparks, E. E. (2024). Root responses to abiotic stress: a comparative look at root system architecture in maize and sorghum. *Journal of experimental botany*, 75(2), 553–562. <https://doi.org/10.1093/jxb/erad390>
- Javaux, M., Schröder, T., Vanderborght, J., & Vereecken, H. (2008). Use of a three-dimensional detailed modeling approach for predicting root water uptake. *Vadose Zone Journal*, 7(3), 1079-1088. <https://doi.org/10.2136/vzj2007.0115>
- Leitner, D., Klepsch, S., Bodner, G., & Schnepf, A. (2010). A dynamic root system growth model based on L-Systems. *Plant and Soil*, 332(1), 177-192. <https://doi.org/10.1007/s11104-010-0284-7>

- Lobet, G., Pagès, L., & Draye, X. (2011). A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant physiology*, 157(1), 29–39. <https://doi.org/10.1104/pp.111.179895>
- Lobet, G., Pagès, L., & Draye, X. (2014). A modeling approach to determine the importance of dynamic regulation of plant hydraulic conductivities on the water uptake dynamics in the soil-plant-atmosphere system. *Ecological Modelling*, 290, 65–75. <https://doi.org/10.1016/j.ecolmodel.2013.11.025>
- Lynch, J. P., Nielsen, K. L., Davis, R. D., & Jabllokow, A. G. (1997). SimRoot: Modelling and visualization of root systems. *Plant and Soil*, 188(1), 139–151. <https://doi.org/10.1023/A:1004276724310>
- McGraw-Hill. (2003). McGraw-Hill dictionary of scientific & technical terms (6th ed.). McGraw-Hill.
- National Research Council (US) and Institute of Medicine (US) Panel on Needle Exchange and Bleach Distribution Programs; Normand, J., Vlahov, D., & Moses, L. E. (Eds.). (1995). Preventing HIV Transmission: The Role of Sterile Needles and Bleach (Chapter 6: The Effectiveness of Bleach as a Disinfectant of Injection Drug Equipment). Washington (DC): National Academies Press (US). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK232358/>
- Paez-Garcia, A., Motes, C. M., Scheible, W. R., Chen, R., Blancaflor, E. B., & Monteros, M. J. (2015). Root Traits and Phenotyping Strategies for Plant Improvement. *Plants* (Basel, Switzerland), 4(2), 334–355. <https://doi.org/10.3390/plants4020334>
- Pagès, L. (2000). Why model root system architecture? In A. Stokes (Ed.), *The supporting roots of trees and woody plants: Form, function and physiology* (pp. 187–194). Springer Netherlands. https://doi.org/10.1007/978-94-017-3469-1_18

- Pagès L. (2011). Links between root developmental traits and foraging performance. *Plant, cell & environment*, 34(10), 1749–1760. <https://doi.org/10.1111/j.1365-3040.2011.02371.x>
- Pagès, L., Bécel, C., Boukcim, H., Moreau, D., Nguyen, C., & Voisin, A.-S. (2014). Calibration and evaluation of ArchiSimple, a simple model of root system architecture. *Ecological Modelling*, 290, 76-84. <https://doi.org/10.1016/j.ecolmodel.2013.11.014>
- Pagès, L., & Kervella, J. (2018). Seeking stable traits to characterize the root system architecture. Study on 60 species located at two sites in natura. *Annals of Botany*, 122(1), 107–115. <https://doi.org/10.1093/aob/mcy061>
- Pagès, L., Moreau, D., Sarlikioti, V., Boukcim, H., & Nguyen, C. (2012). ArchiSimple: A parsimonious model of the root system architecture. In 2012 IEEE 4th International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications (pp. 297-303). <https://doi.org/10.1109/PMA.2012.6524849>
- Pagès, L., Vercambre, G., Drouet, J.-L., Lecompte, F., Collet, C., & Le Bot, J. (2004). Root Typ: a generic model to depict and analyse the root system architecture. *Plant and Soil*, 258(1), 103-119. <https://doi.org/10.1023/B:PLSO.0000016540.47134.03>
- Passot, S., Gnacko, F., Moukouanga, D., Lucas, M., Guyomarc'h, S., Ortega, B. M., Atkinson, J. A., Belko, M. N., Bennett, M. J., Gantet, P., Wells, D. M., Guédon, Y., Vigouroux, Y., Verdeil, J. L., Muller, B., & Laplaze, L. (2016). Characterization of Pearl Millet Root Architecture and Anatomy Reveals Three Types of Lateral Roots. *Frontiers in Plant Science*, 7. <https://doi.org/10.3389/fpls.2016.00829>
- Passot, S., Couvreur, V., Meunier, F., Draye, X., Javaux, M., Leitner, D., Pagès, L., Schnepf, A., Vanderborght, J., & Lobet, G. (2018). Connecting the dots between computational tools to analyse soil-root water relations. **Journal of Experimental Botany.** Advance online publication. <http://dx.doi.org/10.1093/jxb/ery361>

- Penn State College of Agricultural Sciences. (n.d.). Common Bean Shovelomics. Retrieved December 5, 2023, from <https://plantscience.psu.edu/research/labs/roots/methods/field/shovelomics/intensive-bean-crown-phenotyping>
- Postma, J. A., Kuppe, C., Owen, M. R., Mellor, N., Griffiths, M., Bennett, M. J., Lynch, J. P., & Watt, M. (2017). OpenSimRoot: widening the scope and application of root architectural models. *The New phytologist*, 215(3), 1274–1286. <https://doi.org/10.1111/nph.14641>
- Salaspuro M. (1997). Microbial metabolism of ethanol and acetaldehyde and clinical consequences. *Addiction biology*, 2(1), 35–46. <https://doi.org/10.1080/13556219772840>
- Schnepf, A., Leitner, D., Landl, M., Lobet, G., Mai, T. H., Morandage, S., Sheng, C., Zörner, M., Vanderborght, J., & Vereecken, H. (2018). CRootBox: a structural–functional modelling framework for root systems. **Annals of Botany*, 121*(5), 1033–1053. <https://doi.org/10.1093/aob/mcx221>
- Seethepalli, A., Dhakal, K., Griffiths, M., Guo, H., Freschet, G. T., & York, L. M. (2021). RhizoVision Explorer: open-source software for root image analysis and measurement standardization. *AoB PLANTS*, 13(6), plab056. <https://doi.org/10.1093/aobpla/plab056>
- Seethepalli, A. and York, L. M. (2020). RhizoVision Explorer - Interactive software for generalized root image analysis designed for everyone (Version 2.0.3). Zenodo. <http://doi.org/10.5281/zenodo.4095629>
- Spek, L. Y. (1997). Generation and visualization of root-like structures in a three-dimensional space. **Plant and Soil*, 197*(1), 9–18. <https://doi.org/10.1023/A:1004236626479>

- Storey, A. (2016, August 22). Pros and cons of perlite in hydroponics: Is it for you? Upstart University. Retrieved November 7, 2023, from <https://university.upstartfarmers.com/blog/perlite-in-hydroponics>
- Trachsel, S., Kaeppler, S. M., Brown, K. M., & Lynch, J. P. (2011). Shovelomics: High throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*, 341(1-2), 75-87. <https://doi.org/10.1007/s11104-010-0623-8>
- Trees.com Staff. (2023, April 13). What is vermiculite? What is it used for? Trees.com. Retrieved November 7 2023, from <https://www.trees.com/gardening-and-landscaping/vermiculite>
- Vinutha, H.P., Poornima, B., Sagar, B.M. (2018). Detection of Outliers Using Interquartile Range Technique from Intrusion Dataset. In: Satapathy, S., Tavares, J., Bhateja, V., Mohanty, J. (eds) *Information and Decision Sciences. Advances in Intelligent Systems and Computing*, vol 701. Springer, Singapore. https://doi.org/10.1007/978-981-10-7563-6_53
- Wu, L., McGechan, M. B., McRoberts, N., Baddeley, J. A., & Watson, C. A. (2007). SPACSYS: Integration of a 3D root architecture component to carbon, nitrogen and water cycling—Model description. *Ecological Modelling*, 200*(3–4), 343-359. <https://doi.org/10.1016/j.ecolmodel.2006.08.010>
- Yamaguchi (2002) Measurement of root diameter in field-grown crops under a microscope without washing, *Soil Science and Plant Nutrition*, 48:4, 625-629, DOI: 10.1080/00380768.2002.10409248

Appendix

Réactifs		Solution mère		Solution fille		
Composants	M.M (g/mol)	[C] (M)	[C] (g/L)	V/L (ml)	V/25L (ml)	[C] finale dans 25L
KNO ₃	101,103	2	202	2,5	62,5	0,005
Ca(NO ₃) x 4H ₂ O	236,15	2	236	2,5	62,5	0,005
MgSO ₄ X 7H ₂ O	246,47	2	493	1	25	0,002
NH ₄ NO ₃	80,04	1	80	1	25	0,002
Minors :						
H ₃ BO ₃	61,83	0,0463	2,86			0,000046
MnCl ₂ X 4H ₂ O	197,91	0,0091	1,81			0,000091
ZnSO ₄ X 7H ₂ O	287,56	0,0008	0,22	1	25	0,00000076
CuSO ₄ X 5H ₂ O	249,69	0,0002	0,051			0,00000031
H ₃ MoO ₄ X H ₂ O						
or	180,97	0,0005	0,09			0,00000049
Na ₂ MoO ₄ x 2 H ₂ O	241,95	0,0005	0,12			0,00000049
KH ₂ PO ₄ (pH to 6 with 3M KOH)	136,08	1	136	0,5	12,5	0,0005
C10H12N2FeNaO8	421,1	0,0356	15	3	75	0,0001068

Figure 49: Hoagland composition (ELIA protocole PEPA021)

Quantification of plant Root architecture: development of an integrated experiment-to- model pipeline

Presented by Mariam Kerdoudi

Root system architecture is vital for plant growth and stress resistance but challenging to study due to soil development and root fragility. This research aims to enhance data acquisition methods for better modeling.

Protocols were established to streamline experiments, and two root analysis tools were introduced: SmartRoot (semi-automated) and RhizoVision (fully automated). These tools provide data on root characteristics, which are analyzed using the ArchiSimple model.

ArchiSimple, a C++ model, requires 24 parameters to simulate root growth. An R script was developed to obtain five key parameters (Dmin, Dmax, CVDD, DIDm, and IBD) from SmartRoot data, which were validated against existing research.

The study emphasized the need for detailed statistical analysis to ensure accurate conclusions. While the R script saves time, it must be used correctly and adapted to specific data.

Combining SmartRoot and RhizoVision offers a complete understanding of root structure, as each has unique strengths. Future work should integrate the remaining parameters into the script and gather more comprehensive data.