# How to digitalized an entire maize root system to create a R-SWMS input file

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# Contents

# Chapter 1

# Introduction

## 1.1   General principle

The general principle of this method is to acquired an R-SWMS input file from a rhizotron grown plant. The method is divided in three steps:

1. Scan the root system

2. Digitalize the roots

3. Create the input file

Once the input file is created it be directly used as an input file for the R-SWMS model [2].

We tested the method with 15-days old maize plants. These plants presented several advantages for the methods:

- they had thick roots

- they did not have too much roots

- but they already had all type of roots (principal, seminal, crown and laterals)

The method might not be suitable for plant having thinner roots or for older plants as the manipulation time required for some of the steps might increase dramatically.

## 1.2   Required material

| | |
|---|---|
| **Root drawing:** | transparent film |
| | permanent marker |
| | tape |
| **Root scan:** | flat-bed scanner |
| | scissors |
| **Root digitalization:** | ImageJ |
| | SmartRoot |
| | SQL database (Access or MySQL) |
| **R-SWMS input file creation:** | SRToRSWMS.jar |

# Chapter 2

# From a rhizotron grown plant to scanned roots

## 2.1 Principle

Two type of root images can be obtained from a rhizotron experiment: hand drawing and scanning. The had drawing had the advantage to conserve the geographic information of the root system, but present a poor level of detail. On the other hand, the root scan shows all the roots, but not in the same position as they were in the rhizotron. More over, a scan of a complete root system usually present a lot of overlapping region, leading to bias in the root system analysis.

The method we propose used these two types of image at the same time to acquire a geographically correct, highly detailed root dataset:

1. Trace the root axis on a transparent film

2. Fix the film with the drawing at the bottom of the scanner

3. Take the root system out of the rhizotron, clean it and separate the different root axis

4. Replace the cut axis on the drawing fixed in the scanner and scan them individually

This method generate on root scan per root axis, with a correct geographic position and a high level of detail.
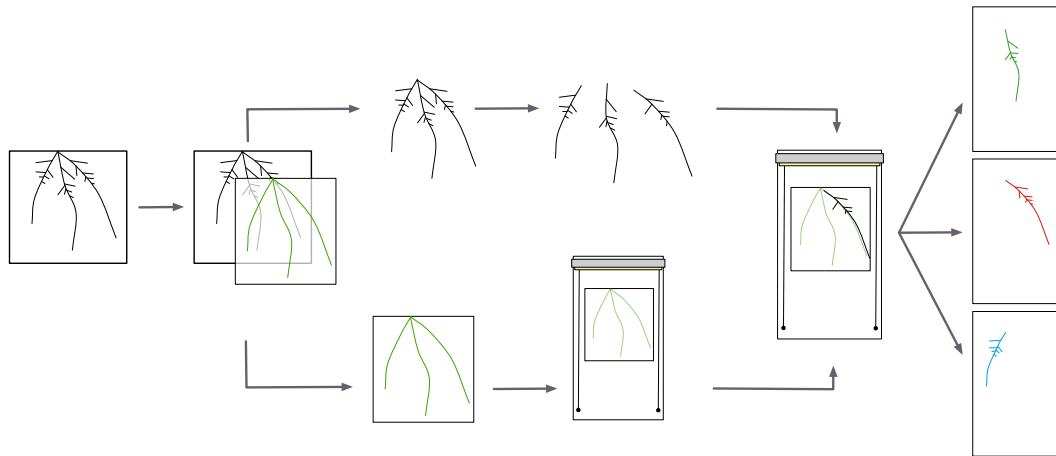


Figure 2.1: **From a rhizotron grown plant to scanned roots.**

## 2.2 Plant culture

Plant were grown in thin transparent boxes (rhizotron, fig. 2.2) during two weeks. At this time the plants reached the fully expanded leaves stage. They root systems had principal, seminal and crown axes (naming according to [1]) and had a maximal depth between 300 and 500 mm.
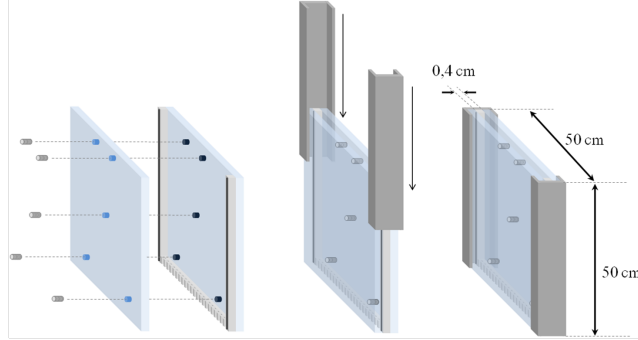


Figure 2.2: **Rhizotrons.** The rhizotron had two transparent sides and an internal volume of 4 x 500 x 500 mm$^3$

## 2.3 Tracing the root axis

Before tacking the plant out of the rhizotrons, draw on a transparent film the different root axis (fig. 2.3).

For this, it is important to use a color that will not be seen on the root scan (as the drawing will be scanned with the root). Use light colors such as green or yellow, and avoid colors such as red of blue.
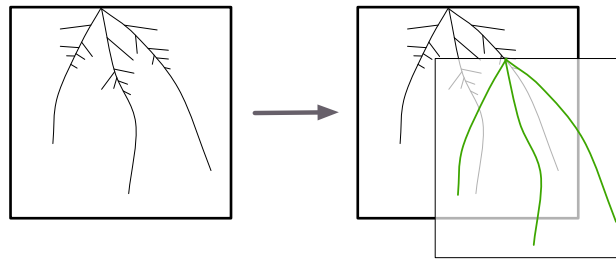


Figure 2.3: **In situ drawing.** Drawing of the axis of the root system inside the rhizotron

## 2.4 Preparing the roots

Take the roots out of the rhizotrons and wash them carefully. A soap bath and a brush can be used to ease the cleaning. If you do not want to directly scan your root system (for instance if you have several of rhizotron to unmount), store them either in clean water (for a few hours) or in FAA (for weeks if needed).

## 2.5   Individual root scan

Once the tracing is done, fix the root axis drawing at the bottom of the scanner (fig. 2.4). It is better to use a flatbed scanner in which the roots are sitting in the water (fig. 2.5) in order to allow them to recover their initial shape.

For every root system you want to scan, cut the different root axis. For each of these root axis, replace it on the root drawing and scan it individually (fig. 2.4). Because every root axis is different and because they tend to recover their initial shape in the water, replacing an axis on its drawing is relatively easy. Usually, lateral roots will keep their initial shape and angle in the water. If they are tangled, do not hesitate to use a brush the separate them. try to avoid root overlapping as much as possible.

For an optimal scanning procedure, use a 600 DPI resolution.

Be careful not to move the root axis drawing fixed at the bottom of the scanner. It is crucial that it does not move.

A the end of this procedure, you will end up with several $x$ scans by root system, $x$ being equal to the number of root axis (fig. 2.4).
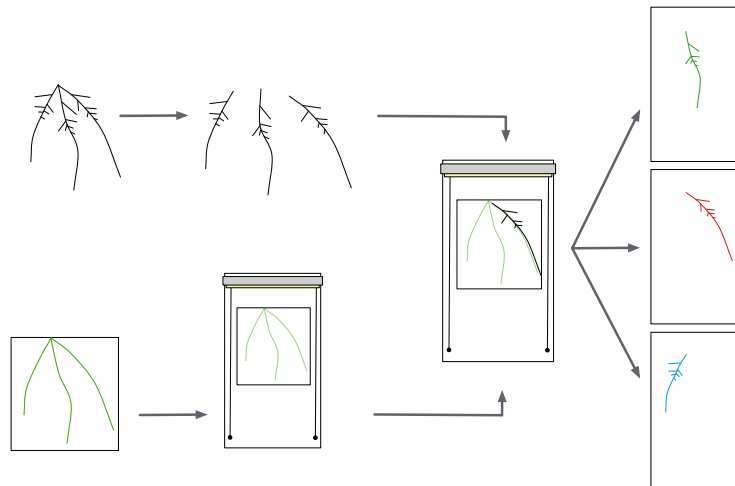


Figure 2.4: **Individual root scan.** First the root axes drawing is fixed at the bottom of the scanner. Then, every root axes is replace on the axes drawing as it was inside the rhizotron and scanned separately.
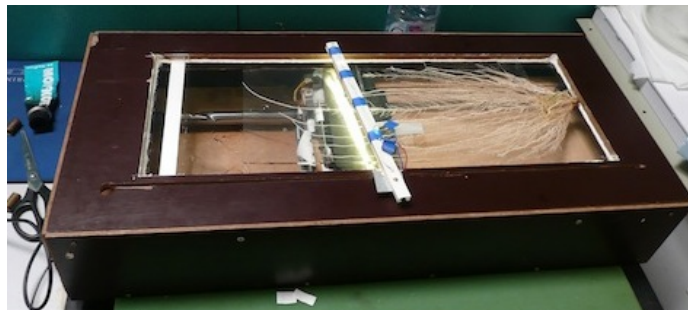


Figure 2.5: **Root scanner** The scanning window can be submerge with water in order to easily spread the roots.

# Chapter 3

# From scanned roots to digitalized structure

## 3.1 Principle

Now that the roots are scanned, we would like to extract useful topological and morphological informations out of the scan. We also would like to be able to create a digital version of the root system.

To achieve these two goals, every root system will be analyzed with ImageJ [**?**] and SmartRoot [3]. The results of the different racing will be send to an unique data table (one data table per root system) (fig. 3.1).
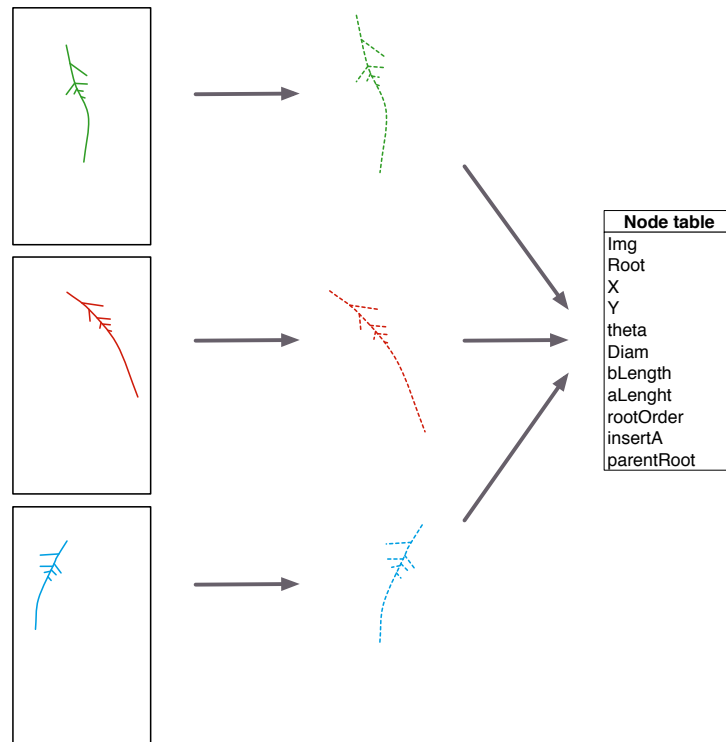


Figure 3.1: **Root numerisation.** Trace every axes with SmartRoot and export all the node data to a single data table.

## 3.2    Before tracing the roots

Because the scan image size is not always the same as the size of the root drawing we will have to crop the different root scan before analyzing them.

1. Open ImageJ

2. Go to `File > Import > Image Sequence` and choose the first scan of the root system

3. Make a rectangle selection which encompass the whole root drawing with top of the rectangle at the top of the drawing (fig. 3.2).

4. Crop the image sequence (`Image > Crop`)

5. Separate the sequence into individual images (`Image > Stack > Stack to Images`) and save them.
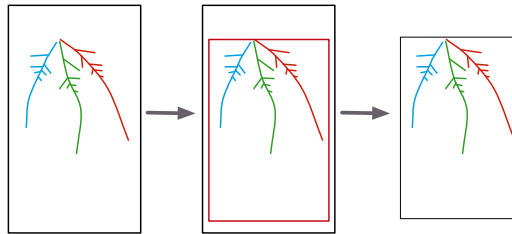


Figure 3.2: **Image crop** Make a rectangle selection which encompass the whole root drawing with top of the rectangle at the top of the drawing. Crop the image image sequence

## 3.3    Tracing and exporting the roots

Open SmartRoot and start tracing the root. For instruction about how to use SmartRoot, please refer to the user manual.

Some recommendations / tips for the tracing:

- Consider the root axis as the parent

- Do not forget topology (attach laterals to their parent)

- Use the `Fast find Laterals` function to automatically trace lateral root.

- For manually traced laterals, use the `Root List` panel to attach them to their parent

One the root is traced, export it to your database (using the `Data transfers` pane).

If you are exporting the first tracing of a root system, you have to create the data table. To do so, write down its name, check the `Create a new table` button and select the `Root Nodes` dataset. It is important that the name is in the form **EXP$x$_R$y$_N**, where $x$ is the identifier of the experiment and $y$ the identifier of the rhizotron (has to be an integer). Click the `Transfers` button to create the table and export the data

For the next tracing, use the same dataset and the same table name but uncheck the `Create a new table` button. If this button stay checked, the new export will erase the previous ones.

# Chapter 4

# From digitalized structure to R-SWMS input file

Once the root system is digitalized, it is possible to retrieve the digitalized data and to use them to generate an R-SWMS input file. A small program, SRToRSWMS, was created to generate these input file. This program can generate the input files for all the rhizotron of one experiment at the same time.

## 4.1   How to use the software

- If you are on Windows, right click on the SRToRSWMS_win.jar file and choose `Open with > JavaTM (SE)..`

- If you are on MacOS, double click on the SRToRSWMS_mac.jar file.

- A new window will open

- Fill the different fields:

  **Experience number:** Your experience identifier

  **Number of rhizotrons:** The number of rhizotrons in your experiement

  **Mean growth rate:** The mean root growth rate in cm/day

  **Driver:** the database driver name

  > **Microsoft Access:** sun.jdbc.odbc.JdbcOdbcDriver
  > **MySQL:** com.mysql.jdbc.Driver

  **Connection:** The connection info

  > **Microsoft Access:** jdbc:odbc:
  > **MySQL:** jdbc:mysql://localhost/

  **Name:** The database name

  **Username:** The connection username

  **Password:** The connection password

  **Output options:** Choose the output folder for the R-SWMS input files.

- Hit the `Run` button

# Bibliography

[1] F Hochholdinder, W J Park, M Sauer, and K Woll. From weeds to crops: genetic analysis of root development in cereals. *TRENDS in Plant Science*, 9(1):42–48, 2004.

[2] M Javaux, T Schröder, Jan Vanderborght, and Harry Vereecken. Use of a three-dimensional detailed modeling approach for predicting root water uptake. . *Vadoze Zone Journal*, 7:1079–1088, 2008.

[3] Guillaume Lobet, L Pagès, and X Draye. A Novel Image Analysis Toolbox Enabling Quantitative Analysis of Root System Architecture. *Plant Physiology*, 157:1–11, July 2011.