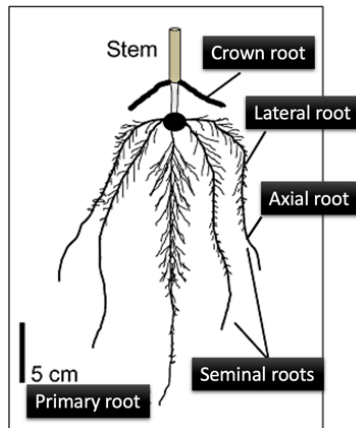




Root Phenotyping Protocol



Root Phenotyping System

Video workshop:

<https://www.youtube.com/watch?v=DxHnbwvSGw>

Software:

<https://zenodo.org/record/4095629#.X7Fi5tNKhBw>

Paper:

<https://spj.sciencemag.org/journals/plantphenomics/2020/3074916/>

Keeping track of your plants

It is very important that the stem portion of each plant can be connected back to the root portion of the plant. Therefore, each plant should receive an identification number. When you separate the stem and shoot portion, remember to label carefully so that you know which root system belongs with which shoot system downstream.

Washing roots

Course washing: Wash your root system until they are relatively clean of the growing medium. Keep each plant in its own properly labeled bag so that the root system never dries out because this will affect root measurements later on. Immediately freeze the roots until you are ready to work on them.

Fine washing: Suspend the root system in a tub of water. Use a pressurized sprayer hose to carefully spray the roots to dislodge bits of media. Be careful not to break the roots. Collect any root pieces that might have broken off during the process, and make sure that you are able to tell where the broken pieces came from (primary root, seminal roots, crown roots). If you cannot tell, then discard them. Do not use those root pieces for scanning.

Sectioning: While your root system is suspended in water, use a pair of scissors or razor blade to cut the root system into the primary root, seminal roots, and crown roots. Cut as close to the seed or stem as possible. Carefully tease these apart so that you don't break any roots. You do not need to keep any parts of the plants that are not the actual roots.

Mounting: Make sure the bottom side of your scanning tray is dry. Pour a thin layer of water into your mounting tray (the water does not have to cover the bottom of the tray). Spread the root system onto the tray as much as possible, avoiding the center circle of the tray. Use plastic forceps and probes to manipulate the root pieces.

To scan, bring the tray to the scanner and gently pour water into one corner of the tray until the roots are completely submerged. Readjust any roots that have been moved. A plastic pipet can help you gently move water to and from the tray.



Scanner Care

Please take care of all aspects working with the scanners.

- **IMPORTANT:** Immediately wipe off any water that got onto the scanner. This is essential to keep the scanner from shorting out.
- When working with the lid, make sure that it sits mostly flat on the scanning tray.
- Be gentle when setting your trays or the lid to avoid cracking the glass.

Epson v850 scanner parameters

Note: The white background panel is used only for leaf scanning. Be sure to remove it before scanning. There is no need for a ruler scale - the software we will can calculate the scale values from the resolution of the image.

Scanner parameters should be adjusted to as follows:

- Scan Settings: Maize Root Phenotype (if not available, set the parameters as below and save using this name)
- Document Type: Film (with Film Area Guide)
- Film Type: Positive Film
- Image Type: 16-bit Grayscale
- Resolution: 1200 dpi
- Image Format: JPEG > Options > Image Quality: 1 (High Quality)
- File Name: provide a name that identifies the inbred line, replicate plant, root type (Primary root, Seminal Roots, Crown roots)

For consistency, please use the following naming convention:

inbred-plantNumber-rootType-imageNumber.jpg

inbred = the inbred name

plantNumber = the number that you have assigned each of your plant

rootType = primary, seminal, crown

imageNumber = you will likely need to scan multiple seminal and crown roots, so this will help you keep track of them (the scanner only allows you to use 3 digits, so start with 001)

Scan a preview to see your root system. Then select the area(s) that you'd like to scan. Scan each of your root types (primary root, seminal root, crown root) as an individual image. The resulting scans produce an image for each selection. You may scan multiple seminal or crown roots in one scan or you may separate them, depending on the size of your root system and your preference.

Save your scanned images into your own folder under the [Root Phenotype](#) shared folder. Take care to only edit your own folder and files. Do not edit anyone else's data.

Before moving on, make sure the images are named correctly. Make any corrections as necessary.



RhizoVision Explorer software parameters

Image pre-processing

- Analysis mode: **Broken roots** (for multiple scanned root pieces)
- Convert pixels to physical units: check box, and type in the PDI that you scanned the roots (usually **1200 dpi**).
- Image thresholding level: this will vary for each scan but will be **around 240**. Play around a bit until you are satisfied that the threshold is strong enough to capture contiguous roots in your system.
- Filter non-root objects: check box. Maximum size (mm): This will depend on each scan. Keep adjusting the number until you feel like you have a nice and clean root image.
- It is unlikely that you will need to use the “Fill holes in root objects” option, but may be required for some scans.
- Enable edge smoothing: check box. Set to 2. This option will help improve your segmented image.

Feature extraction

- Enable root pruning: Always use this option
 - Root pruning threshold: set to 5
- Root diameter ranges: we will use three root diameter ranges
 - Range #1: 0-0.3 (2nd order roots)
 - Range #2: 0.3-1.0 (lateral roots = 1st order roots)
 - Range #3: 1.0 and above (axial root)

Saving data

IMPORTANT: Save your data after the processing of each image/region of interest into your [Root Phenotype](#) shared folder. The software crashes all the time (very typical of root phenotyping software) so make sure that you save after each run to prevent loss of data.

- Once you have finalized the parameters that you deemed best for your root system, clear all the rows in the “Features” panel, then run the analysis one more time.
- Save the features by going to File > Features. Name your root system appropriately so that you know which inbred, plant, and root type the data belongs to.

Batch processing

Use batch processing mode to process all images stored in a folder. It is very important that you decide on which parameters work best for your inbred. Once you have figured out the best parameters that describe the three root ranges, apply those parameters in batch processing mode.

Batch processing mode is *not* recommended unless you have a lot of photos that need to be processed. You gain time but lose data resolution.

- Place all the photos you’d like to process into a folder
- Set your **Image pre-processing** to the proper parameter as described above
- Set your **Feature extraction** according to your root phenotype
- Go to File > Batch analysis
- Image(s) location: select the folder where images are stored



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- Output location: select the folder where you'd like the results to be stored
- Rename output files if you wish. These will be csv files for your extracted features as well as parameters that you used to run the batch so that you can run them again as needed.

Combine final data

Once you have processed all of your images, combine the data into a single spreadsheet so that you can process your raw data in R.

Got an amazing root scan?

We pick out some nice root scans at the end of the program for posters, cards, etc...If you've got some amazing ones, make good notes so that you can find them again.