

Root & Nodule Quantification Software

INSTRUCTIONS FOR IMAGING ROOT SYSTEMS

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INTRODUCTION

This software was developed for recording and quantifying nodule distribution patterns in a standardized way, based on image analysis. The software can be used to quantify differences in nodulation patterns among cultivars, under different environmental conditions, and/or between different legume species. Such quantitative phenotyping will make it possible to explore the link between nodulation patterning, root system architecture, and ultimately plant yield.

Please refer to the associated publication for more information. This publication contains essential information to understand how the images need to be taken, how the software works, and what data are extracted.

Please carefully read the Instructions for Imaging Root Systems for Root & Nodule Quantification Software before starting your experiment for important details on imaging your plants appropriately for the software. If these instructions are not followed carefully, the software will not work optimally, or may result in errors.

ROOT SYSTEM PREPARATION

MATERIALS

- 8 x 10 inch square of black felt
- Large square Tupperware container
- Tweezers
- Scissors
- A 1.5 inch long rectangle of Styrofoam
- Dissecting pin
- Camera
- Level

INSTRUCTIONS

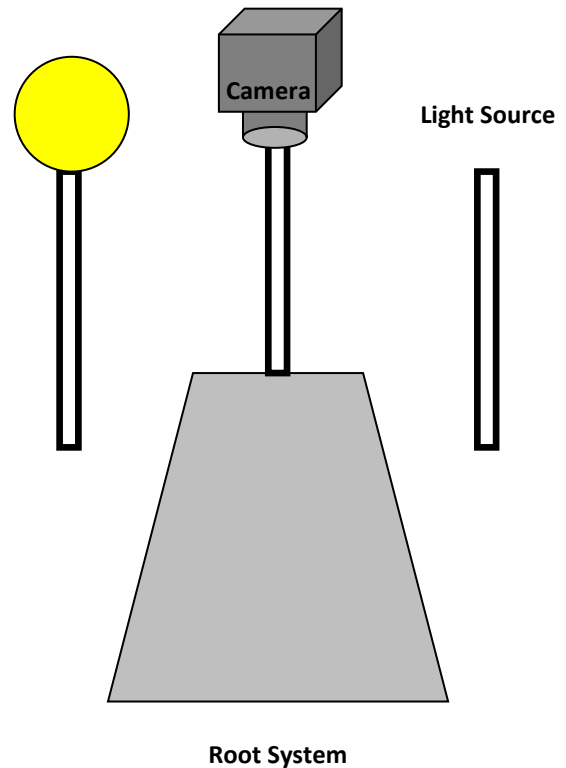
1. Place the root system of your plant under running water to clean away all soil and debris from the roots
2. Using scissors remove the plant's shoot directly above the cotyledons
3. Fill up the Tupperware container with water and float the root system in it, removing all tertiary lateral roots from the root system with tweezers at their base (floating the roots in water makes this step much easier)
4. Wet the felt and center the entire root system on it, the felt should be in a landscape orientation with the primary root horizontal along the top of the long axis with the cotyledons in the top right hand corner (this orientation applies to all photographs and is crucial for the imaging software to work properly)
5. Straighten the primary root as much as possible
6. Indicate the tip of the primary root with a dissecting pin
7. Label the 1.5 inch Styrofoam marker with a 3 letter identifier (eg. SPK for Sparkle), the plant number (eg. 001) and the number of days after inoculation (eg. 28) – SPK 001-28
8. Place the marker on the felt beside the plant so that it is not touching any roots
9. Take a picture of the whole plant (this will be your P0 image when using the Root Nodule Software) see imaging instructions

10. Place the root system back into the water and separate out one plane of roots from the others
11. Place the plant on the felt with the separated plane to one side of the primary root and the roots of the other planes to the other side and cover them with a wetted paper towel
12. Use your tweezers to separate the roots of the isolated plane so that they are not touching one another and the nodules from adjacent roots are also not touching (you only need to do this for the lateral roots within the nodulation zone)
13. Try to straighten the primary root and lateral roots as much as possible
14. Take a picture once again, indicating the tip of the primary root with a dissecting pin and including the 1.5 inch label to the side of the root system (this will be your P1 image)
15. Once the image has been obtained use scissors to remove all lateral roots of that plane as close to the primary root as possible
16. Now you are ready to image the next plane, follow steps 10-14 for both of planes 2 and 3 (your P2 and P3 images)

IMAGING INSTRUCTIONS

For best photographing of roots, follow these instructions:

1. Place the root system to be imaged on a flat surface directly below a mounted camera so that the camera lens is parallel to the root system (use a level to adjust camera position to assure that it is parallel)
2. Adjust the height of the camera above the root system so that the entire root system is in the image if you want to know the length of the primary root, or so that the entire nodulation zone is in the image if you are not concerned with the primary root (once you set the camera position for the first image of a plant e.g. P0, do not move it at all while imaging any other planes of the same plant this will be crucial once you are running the software later)
3. Turn off the flash on the camera and light the root system using two external light sources placed on either side of the root system and angled down
4. Turn off all other light sources in the area where the photograph is being taken
5. Put the camera on a manual setting and adjust exposure settings so that there is enough light that even very fine roots are visible in the image, but not so much that there is a lot of glare from the wet roots or felt (we found that exposure settings of 1/10, 1/8, 1/6 worked best)
6. You may want to take multiple images of each plane on different exposure settings, and then decide later which one will work best with the software



Once the photograph is taken you should crop away all non black background using a photo editing program. It is important that the plant is completely on a background of black for the programs to work. If you need to, crop the images to remove everything but the black background that the root system was photographed on.

SAVING YOUR IMAGES

JPG IMAGE FILE NAMING

The software will read the image names in the following way – it is therefore essential that images are named in *exactly* this convention.

XXX ###-## P#.jpg

For example:

WTS 031-28 P1.jpg

- WTS** : 3-letter code for the **group** (genotype / treatment, etc)
- 031** : 3-digit unique **identifying** number for each plant in this group
- 28** : 2-digit **age** of the plant at the time of imaging (DAI or DAP)
- 1** : 1-digit ID for the three **planes** of each plant (P0 for image of whole plant)

FOLDER HIERARCHY

The programs will read through folders in a given hierarchy and therefore requires that your images are saved in a specific way, as follows.

You can save the main project folder anywhere you like (ideally on the C-drive).

Within the main project folder you must create a folder named Images.

...\Main project folder\Images\XXX ###-##

The main project folder can be named anything you want.

I.e. you will have a folder for each plant. Within each folder you will have the 4 jpeg images of that plant.