Leaf Movement Analysis

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Plant Growth

1. Fill the desired number of pvc pipe tubes with germination mix soil such that the soil is completely compressed in the tube.
2. Flatten the bottom of the tubes and make the top of the tubes slightly rounded above the tube rim with soil. It is best to do this using very wet soil.
3. Plant one seed into each pvc pipe tube
4. Allow them to grow for 7-9 days (for Arabidopsis, shorter for Brassica) in a humid environment.
5. Once the seedlings are established, thin the plants such that there is only one seedling in each tube.

Plant Arrangement

1. **Create a map:** To accurately record which genotypes/ treatments are in which position in the images, a system of letters and numbers is generally followed. 120 Arabidopsis and 60 *Brassica rapa* plants fit into one camera set-up so the racks are labeled A-F, from top to bottom, and the plants are labeled 1-20 from left to right. Using this method, a map needs to be made before the arrangement of plants can be done.
2. **Arrange the tubes:** Once the arrangement plan is determined, put the tubes in the racks such that the two cotyledons are pointed out to the sides, the water slots on the racks are on the far left or far right (if the rack is a 1-10 rack, the water slot needs to be on the far left, if the rack is 11-20, the water slot needs to be on the far right).
3. **Build the stairs:** Next, set up the staircase on top of a black tray and in front of a black poster board and fill in the racks of plants. See example image below.
4. **Water:** Carefully fill each rack with DI water up to just before the rim by pouring into the water slots on both ends.

Imaging

(Based on using a Canon Powershot A2300 or ELPH180 with the Canon Hack Development Kit Intervalometer script)

1. Set the camera up on a tripod or camera stand so that it is parallel with the rack and with all the plants in the field of view.
    a. It is a good idea to take a picture and then look at it on a computer to be sure that all plants are in view.
2. To set the camera, be sure that the memory card is locked and has the correct software loaded onto it.
3. Press the play button
4. Press the menu button
5. Scroll to the bottom of the menu and select firmware update
6. Press Ok
7. Press the play button again, at this point, “Intervalometer”* should appear on the screen

8. Press the shutter button to start the program, a message saying “STARTED” should appear at the bottom of the camera screen.
9. There is a number on the screen like 3xxxM, this indicates the amount of memory left, record this number and check back in a few hours to see if the number has gone down. This is a good sanity check that the program is running correctly and that images are being captured.

*Note: The intervalometer script must be modified to capture images every 1200s (20min).

Analysis

These steps need to be repeated for each camera

1. **Directory Set-up:**
    a. Create a folder inside the Leaf_Movement directory in the Documents folder on the lab mac.
    b. Within this folder, create a folder specific for the experiment, then another folder specific for the camera. For example:
    /Documents/Leaf_Movement/TRiP_yourname/Cool_Experiment_2023/C3
    Within the specific camera folder, create a directory: “input”
2. **Upload Images:** Remove the images from the camera's SD card and put them directly into the “input” folder you just created
3. **Camera Stability Check:** Drag and drop the entire “input” folder into Fiji (ImageJ) and create a quick stop motion video, this is done to check that there were no massive movements of the camera during the experiment.
4. **Crop File Creation:**
    a. Take the final image of the set and drag and drop it into Fiji. Select the Record plug in (Plugins → Macros → Record…)
    b. Select the "Rectangle" to carefully draw a box around each plant starting from the top left corner going across in rows. These squares should only include the plant with **minimal to no soil.** In cases where there is no plant visible, draw a small box in empty space so that there is the correct number of boxes. Each time a square is drawn, a line which reads “makeRectangle(--, –, –, –);” will appear in the Recorder window, this is what you want. Make sure you have one line for each plant.
    c. After all boxes have been drawn, copy everything in the record window to a blank text document in a text editor like Atom or BBedit.
    d. Using find and replace, take out all characters and words that are not the coordinates. The document should have four coordinates, separated by a space, on each line, corresponding to each rectangle drawn in the previous step.
    e. Copy these coordinates and enter it into the second column of a blank Excel spreadsheet. If copied correctly, each set of coordinates should be on its own line.
    f. In the first column, enter “plant_A01 ”, for plant 1 in row A. Then continue the pattern: “plant_A02 ” and so on. Keep both the row and plant position in the

name in case there is a problem with the cropping and you want to refer back to the plant from the original image. Note: The space after the position is required.

g. Next string these two columns together in the third column using CONCATENATE and selecting the cells on the first row in both columns. Drag the formula to the bottom of the spreadsheet to do this for all rectangles. If done correctly, each cell in your third column should read something like “plant_A01 100 150 200 250 ” **If you are running the Matlab version of TRiP you will also need to add the path to the cropped folder (ex: ../Camera1/cropped/plantA01 32 28 140 85)
h. Copy the entire third column and paste it into a new text document. Save as “crop.txt” into the “code” folder created earlier.

5. **Matlab:**
    a. **Running the program (from Matlab console)**
        i. Open the TRiP.m file found in the “code” folder. Once Matlab opens, ensure that the working directory is your code folder for the experiment.
        ii. Create a folder called ‘cropped’ in the working directory (must match the path in the crop.txt file).
        iii. Run the program. Errors are possible, but generally explained through differences in naming of the files. For example, a common error will yell at you about the d2 name, for this, find the d2 definition in the estimateALL.m file (line 18), rename it to match your files, save, and rerun the code.
        iv. Check the cropped folder to make sure you are seeing a new folder for each plant that is populated with images of one plant.
    b. **Results:**
        i. All the data output is found in the “output” folder.
        ii. The main set of data is called ‘model_output.csv’ where the individual period lengths for plants can be found, as well as the r squared value and RAE.
        iii. For each plant the traces of the leaf movement are also provided as ‘.png’ images in this same folder.

6. **Python:** See the README in the PyTRiP directory.
    a. **Installing the program** (only once):
        i. Open a terminal in a directory where you want the program and results Clone the repository
        `git clone https://github.com/joanmanbar/TRiP.git`
        ii. Navigate to the PyTRiP folder
        `cd TRiP/code/PyTRiP/`
        iii. Install the required packages
        `pip install -r requirements.txt`
    b. Run the program (assuming Python 3.5 -or greater- is installed):
        i. Execute the TRiP.py program using Python, providing the full paths to your "input" folder, and to the crop file (if cropping is needed). Specify if

you want to calculate motion (-mt True) or just to fit the models (-mt False -m True). If you are not using all the images in the "input" folder, specify the numbers for the first and last images, not their name. For example, if you have 250 images in your "input" folder but will only use from 20 to 240, your code should look like this:

```
python3 TRiP.py -d ../../input/ -e JPG -c
../../input/crop_coords.txt -mt True -m True -s 20 -f
240
```

c. Results:

i. All the data output is found in the “TRiP/code/PyTRiP/output” folder.

ii. The main set of data is called MODELS_DATA.csv where the individual period lengths for plants can be found, as well as the r squared value and RAE.

iii. For each plant the traces of the leaf movement are also provided in graphs in the “TRiP/code/PyTRiP/output/motion" and “TRiP/code/PyTRiP/output/model" folders

iv. All cropped images can be found in the “TRiP/code/PyTRiP/cropped” folder

NOTE: If cropping was needed, the folder will always be created under "TRiP/code/PyTRiP/". This is also true for the "TRiP/code/PyTRiP/output" folder. Therefore, you must remove the “TRiP/code/PyTRiP/cropped” and “TRiP/code/PyTRiP/output” folders from “TRiP/code/PyTRiP/"if you want to run the program again for a different camera or set of images.

Example Images:

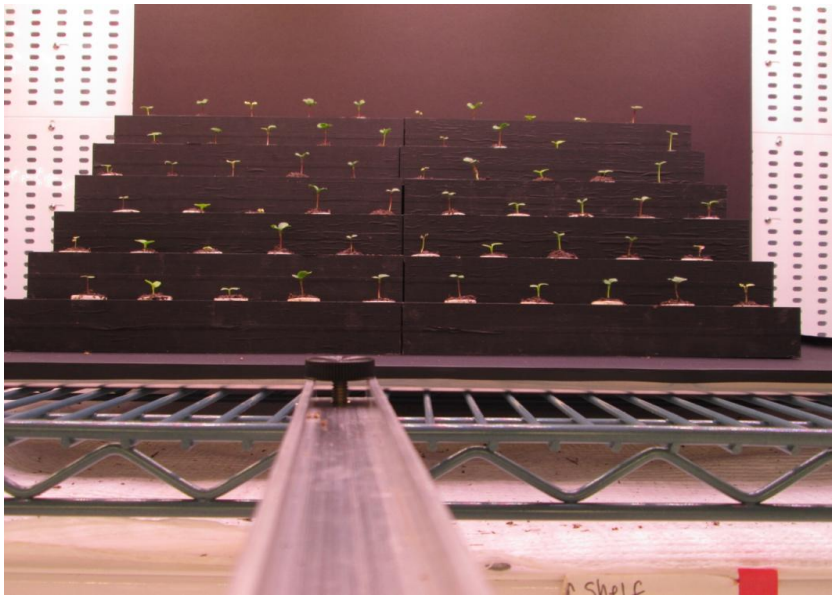

Figure 1: Camera output showing B. rapa plants.

Figure 2: Camera and stand set up