XYLEM PHENOTYPE PROTOCOL, BUERKI LAB, BOISE STATE UNIVERSITY

INTRODUCTION – UPDATED NOVEMBER 2023

Investigating vascular structures in roots can aid in investigating the plant's susceptibility to embolism and ultimately cavitation under drought conditions. This protocol was adapted from common cross sectioning techniques for use with *Artemisia tridentata* seedlings grown under optimum conditions in a greenhouse experiment.

MATERIALS

- Petri dishes.
- Disposable razor blade.
- Toluidine blue dye.
- DI Water.
- Microscope slide & cover slip.
- Kimwipes.

WORKFLOW

1. Prepare root samples by sectioning measured portions of roots. Obtain the first 2 cm of taproot indicated by the first 2 cm below ground. Obtain 3 cm secondary roots from the upper ~50% shooting off taproot. This section should be the 3 cm closest to taproot.

2. Rehydrate roots in DI water, tap roots will take longer for thinner secondary roots should be soaked for ~2 minutes so as not to make them too mushy. If roots are not supple/'snappy', let them dry slightly.

3. Cut cross sections by hand using razor blade. Ensure blade is perpendicular to cutting surface so a straight cut is produced. A fresh edge should be used on each mounting. Thinnest possible cross sections are ideal.

4. Wet mount on slide with square slide cover, ensuring correct orientation of cross sections

5. Add 2-3 drops of toluidine blue (shake bottle first). Orient dropper so dye enters only on one side underneath slide cover.

6. Hold Kimwipe on side opposite to drop placement to pull water out, thereby causing dye to gently permeate throughout slide. Wait 60-120 seconds.

7. Add water under cover slip simultaneously pulling dye out on opposite end of cover slip by using a Kimwipe to absorb liquid out of the cover slip.

8. For taproot: cover slip was removed, each cross section was separated, and standing water was removed using Kimwipes

9. Observe and record images under microscope. Motic Image software was used with Motic Camera and Boreal 2 microscope

DATA PROCESSING

Pulling images for scoring

Prior to downloading images, please refer to DB_xylem database at the following file path: *Buerki_Lab/Stomata. It contains data that links a plant image name with its corresponding unique Plant_ID that other DB's use.

File path: *Buerki_Lab/Stomata/images/RAW_IMAGES/

The RAW_IMAGES folder contains all of the captured images exported from the Motic microscope camera. Each folder is marked with a date (YYYY-MM-DD). Each dated folder contains all the images captured on that specific day.

File path: *Buerki_Lab/Stomata/images/RAW_IMAGES/taprootcomposites

If the photo referenced in DB_xylem contains 'composite' it is located in the taprootcomposites folder in its appropriate plant ID's folder.

How to count xylem and diameter from cross section images:

ImageJ set scale ratio:
@ 40x: 9.2001 / 1u (secondary root xylems)
@ 10x: 2.3033 / 1u
@ 4x: 0.92001 / 1u (tap root xylems)

For secondary root images:
Most secondary roots are small in overall diameter. Xylem are clearly distinguishable as they are the only openings.
All circular openings can be assumed as xylem, and therefore are counted
Diameter should be measured using ImageJ
Measurement lines should be horizontal if possible
For elliptical vessels, measure halfway between longest and shorts chord through the ellipse similar to as follows:

Create a line as above and measure that vector using key command “m”
Save this vector onto the image using key command “ctrl+d”
For macOS devices, the key command is “cmd+d”
Continue to make vector measurements of vessels until all are accounted for on the image
Save the image with measurement lines as a new .jpg’ file and its measurements as a ‘.csv’ file
The names of the files should be the original photo ‘.jpg’ name with an added ‘_measured’ suffix
E.g. ‘20210714_153552_10.jpg’ would have two accompanying files as follows:
‘20210714_153552_10_measured.jpg’
‘20210714_153552_10_measured.csv’
Please upload the completed files into its appropriate plant ID named folder on Google Drive
File path: *Buerki_Lab/Stomata/Secondary/

For tap root images:
Find the appropriate image to measure xylem diameters from DB_xylem
Select a seedling and locate a file with a T1b or a T2b in its ‘Location’ parameter
Download the file and open it in ImageJ
Use the ‘Set Scale’ function (Analyze-->Set Scale) and use the ratio 2.3033 / 1 unit
A sample of xylem will be counted and measured, not all
A 90 degree section of the circle will be sectioned
This should be done using ImageJ with the angle tool
The center of this chunk will be the approximate center of the innermost ring inferred to be the boundary of the first year of growth of the seedling
Prioritize a 90 degree section with the clearest area for identifying xylem diameters
Xylem should be identified as follows:
Each image is stained to a different degree, but xylem in each image should show similar staining
Phloem are more oblong and less circular, and should be avoided
Xylem will be larger than phloem and should take in more dye, indicated by a darker color in comparison to more turquoise phloem
Measure each identifiable xylem using the line tool in ImageJ as outlined in the secondary root image scoring directions above
Save the image with measurement lines as a new .jpg’ file and its measurements as a ‘.csv’ file
The names of the files should be the original photo ‘.jpg’ name with an added ‘_measured’ suffix
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How to measure data on concavities.

Solely for tap root images:
Find the appropriate image to measure xylem diameters from DB_xylem
Select a seedling and locate a file with a T1a or a T2a in its 'Location' parameter
Download the file and open it in ImageJ