Multimodal analysis of plant root composition as a function of drought

Daniela Ushizima[1,2], Esther Singer[3], Elizabeth Carpenter[1], and Dilworth Parkinson[1]

[1] Lawrence Berkeley National Laboratory, Berkeley CA, USA, 94117
[2] University of California Berkeley, Berkeley CA, USA, 94117
{dushizima,esinger,emcarpenter,dyparkinson}@lbl.gov

Abstract. Due to climate change, droughts are increasingly becoming a threat to biofuel crop production. Water limitation can modify plants both phenotypically and chemically. Drought influences root architecture and chemical composition, impacting crop and soil health. Panicum hallii, a biofuel plant model, can be cultivated in a tightly controlled climate chamber called EcoPOD, that allows monitoring and manipulation of interactions among plants, soil, microbes, and atmosphere simultaneously. This work focuses on multimodal imaging of P. hallii with confocal microscopy and microtomography to perform multiscale root analysis. We created exploratory methods to measure root properties in terms of fluorescent intensities for plants treated under different humidity. Preliminary investigations show considerable correlation between cellulose and lignin concentrations in response to drought conditions.

Keywords: confocal microscopy, micro-tomography, biofuel, drought

1 Introduction

In energy sciences, experimental datasets come from diverse instruments, such as those at DOE Nanocenters and X-ray user facilities. In order to establish synthesis-structure and structure-property relationships as part of plant studies, solutions must combine data from different imaging modalities. The need to integrate multimodal multiscale data from complex experiments, such as the analysis of plant roots that have been spatially resolved, is hardly met with protocols that can standardize data across experiments made in the field, or even conducted under simplistic conditions in the laboratory.

For that purpose, EcoPODs are highly controlled climate chambers that bridge reproducible laboratory and complex field experimental platforms, using both above- and below ground sensors for data acquisition about plants through their entire life cycles. Using the EcoPOD, we conducted a drought experiment with a perennial biofuel plant model [1] and collected images from the root to inspect architectural and chemical composition from multiple specimens. In order to do so, we stained complete plant root systems to quantify lignin and cellulose content. While cellulose is the most abundant biopolymer and an important

structural component of the cell walls of plants, lignin is a complex biopolymer that strengthens and waterproofs secondary cell walls, enabling mechanical stability and long-distance water transport [2].

Cellulose and lignin concentrations in plant roots have been of interest because they can have direct impact on plant development and health [3], soil erosion prevention [4], and because they work as proxies for one to determine the rate of the plant decay, and hence, they are used to estimated carbon sequestration rates [5]. Cellulose and lignin concentration changes in roots have been found to be correlated to agricultural management techniques [6] and microbiome colonization [7]. However, we know little about how environmental stress, for example caused by climate change, such as droughts, impact root chemistry and associated consequences for the topsoil. While chemical analysis using matrix-assisted laser desorption/ionization (MALDI), mass spectrometry, and elemental analyzers all provide insightful and complementary datasets, these techniques do not preserve the original architecture of the roots and do not allow for spatially resolved chemical mapping.

This work focuses on developing algorithms for multimodal imaging, which provide quantification of the impact of drought on root chemistry and root architecture context of *Panicum hallii*. We consider two data sources operating at two different scales: confocal microscopy and X-Ray microtomography (XCT). This paper will discuss preliminary results on quantifying phenotypical and chemical variations from multiscale imaging of *P. hallii*. Our results show that we are able to: (a) inspect root architecture using microtomography; (b) access lignin and cellulose content by visualizing colocalization from images acquired with a laser scanning confocal microscope, followed by (c) calculating variation of chemical profiles across samples under different water availability conditions, focusing on the relationship between lignin and cellulose across root tips.

2 Materials & Methods

Panicum hallii var. filipes is a model biofuel crop plant native to the United States with small, sequenced genome and short growth period of approximately 2 months from seed to flowering. This monocotyledonous plant has become an emerging model for genetic studies of agronomic traits in *Panicum sp.*, since it presents a tractable diploid alternative study system to the tetra- or octaploid biofuel crop switchgrass (*P. virgatum*) [8].

This plant variety stems from the mesic parts of Texas, exhibiting delayed flowering and producing clonal seeds. As such *P. hallii* is an ideal genetic model for the biofuel champion switchgrass and other C4 perennial grasses, and it is a favorable plant for EcoPOD experiments, in which we can test the impact of drought on the pre-flowering phase of the plant. Specifically, the compact nature of this plant and its reproducible root branching pattern allow accessing of root hair density and surface area, as well as cellulose-lignin ratios along different root segments as a function of drought stress.

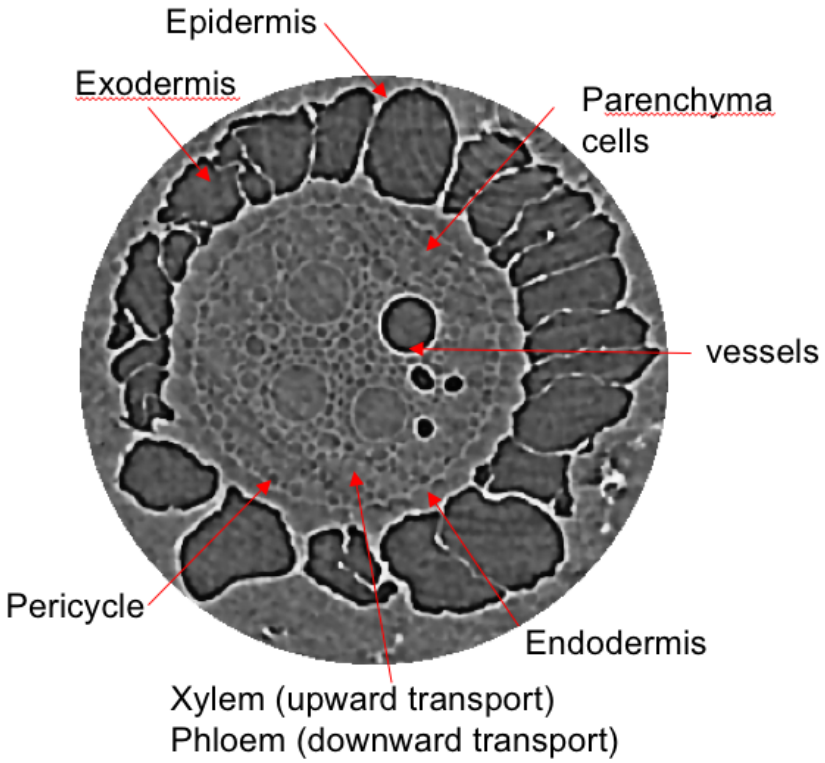

Fig. 1: Transverse cut of *P. hallii*: anatomical plant structures from microCT image slice.

In order to prepare samples for imaging, *P. hallii* seeds underwent vapor-phase sterilization as described in [9]. Seedlings were transferred to agricultural soil from the University of California, Davis, and cultivated at 26^{o}C atmospheric temperature with 12 hour day-night cycles. All seedlings were grown for two weeks in well-watered conditions. Half of the seedlings were drought treated, i.e., irrigation was stopped for five days. All plants were taken out of containers thereafter at 19 days of age. Soil from all roots was cleaned by dipping in DI water for approximately 10 seconds. Roots were then fixated in 4% glutaraldehyde solution in 100 ml PBS for 7 days. Roots were processed for XCT, and for confocal imaging, as described in the next sections.

2.1 Sample preparation for XCT

Stained roots were segmented by root order (1: closest to the crown, 2: 2nd branching order, 3: root branch with root tip) and embedded in 7% agar. Embedded roots were transferred to the micro-CT X-ray facility at the Advanced

Light Source, Lawrence Berkeley National Laboratory, within an hour of preparation. Figure 1 shows a digital slice from a reconstructed volume. The pixel size is approximately 1.6 microns, and brighter intensities correspond to higher absorbing structures. Darkest pixels are often associated to air-filled space. In order to obtain images with sharper borders, intermediate amount of phase contrast was considered during image acquisition, with highest contrast appearing at the boundaries of air spaces. Regions of the sample where there is plant material next to agar have very low contrast, which is sub-optimal, but the resolution would degrade otherwise. Depending on which image features are most relevant, it is possible to adjust the amount of phase contrast to trade off contrast vs. resolution.

2.2 Sample preparation for confocal

Fixated roots were stained using Calcofluor White (10 ml into 100 ClearSee) overnight to visualize cellulose and afterwards with a Basic Fuchsin solution (2 g into 100 ml ClearSee) for 4 hours to visualize lignin [10]. Stained roots were subsequently segmented by root order (1: closest to the crown, 3: root tip) and mounted individually onto microscopy slides in water. These samples were imaged at the Facility for Advanced Microscopy, Lawrence Berkeley National Laboratory using 20X magnification on a ZEN 2.3 SP1 FP2 (Black) (64-bit) using a laser scanning microscope LSM 710, controlled with software release version 14.0.16.201 (Carl Zeiss Microscopy GmbH, Jena, Germany). Images reported here used resolution equal to 2.4089 pixels per micron, with voxel size of 0.4151x0.4151x1 micron^3.

3 Preliminary Results

We analyzed the specimens in Figure 2, which were maintained under controlled conditions, i.e. in a laboratory setting that mimics an environment with complexity similar to the field, with results that are expected to be directly applicable to the field/real-world. Computation work used algorithms for image segmentation as discussed in [11]. In order to improve reproducibility, codes are increasingly becoming available at this github repository[3]. At three weeks old, plants were $\sim$ 20 cm in height with root lengths of $\sim$ 13 cm. Figure 2 (right) shows that drought led to decreased levels of the lignin and cellulose, as emphasized by the two fluorescent signals. According to analyses based on these specimens, we observed a higher cellulose-lignin ratio on the control (left) in comparison with the drought model. Staining illustrated in Figure 2 reveals maxima of fluorescence intensity at the root tip and lattice-like patterning at the boundary to cell walls, which is expected as part of the plant survival mechanism under drought conditions. Figure 3 shows the relative variation of lignin (left) and cellulose (right) for both specimens, with signal peaks of approximately a third of the lignin, and

[3] Source code and documentation for Eyecopod at http://bit.ly/eyecopod

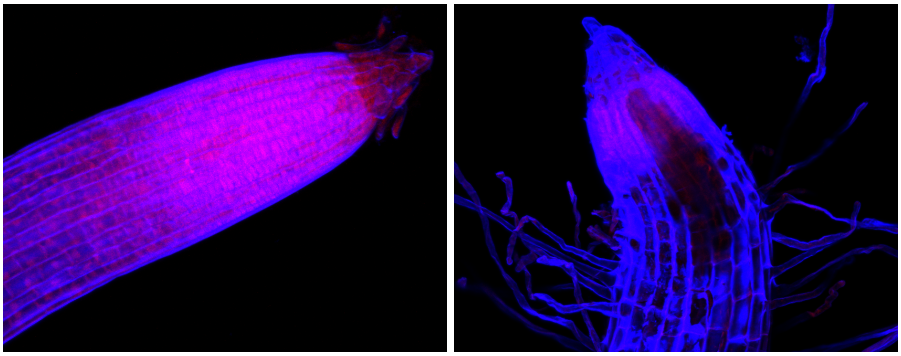

Fig. 2: Longitudinal view of control (left) and drought-regime (right) specimens of P. hallii, both images showing lignin stained with basic fuchsin shown in red tones and cellulose stained with calcofluor white shown in blue tones.

a half of the cellulose expression for the specimen under drought in comparison with control.

These results provide baseline data that demonstrate the applicability of multimodal imaging on root of *P. hallii*, a relevant biofuel crop and drought model. While this study is comprised of samples from only one time point, we plan to expand this study to a full time series that tracks the entire plant growth cycle from seed to flower and along a drought gradient. Thanks to the availability of the EcoPOD, reproducibility is granted and root retrieval is much easier compared to sampling in the field.

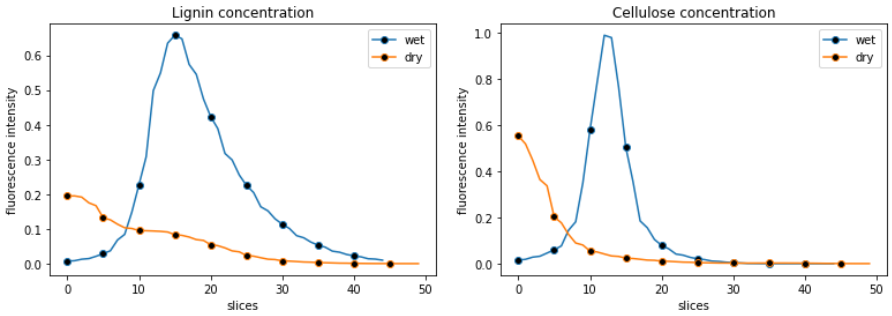

Fig. 3: Longitudinal view of P. hallii control specimen: lignin (left) and cellulose (right) revealing maxima of fluorescence intensity at the root tip.

4 Discussions and Conclusions

This work presents preliminary results on analyzing root architecture using both confocal laser scanning microscopy and XCT. While XCT provided anatomical structure of the roots, the confocal images offered spatially resolved chemical mapping of cellulose and lignin concentrations. These are initial steps toward creating computational tools for quantitative fluorescence colocalization analysis. Future work will include broader inspection of spatial colocalization and chemical correlation across large datasets of biofuel plant roots under drought.

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