Multimodal analysis of plant root composition as a function of drought

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Abstract

Due to climate change droughts are increasingly becoming a threat to biofuel crop production. Water limitation can modify plants both phenotypically and chemically. However, we have limited knowledge on the impact of drought on root architecture and chemical composition, which impact crop and soil health. We cultivated Panicum hallii, a perennial grass, 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 using multimodal experimental data from confocal microscopy and micro-tomography to perform multiscale multimodal root analysis, including measuring fluorescent intensities for plants in different humidity conditions. These measurements rely upon new efforts on computational methods for assessing roots properties and potential functionalities. Preliminary investigations show high correlation between cellulose and lignin concentration in response to drought conditions.

Index Terms

Image analysis, fluorescence colocalization, microtomography, confocal microscopy.

I. 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, solutions must combine data from different sources and science domains. The challenge is to integrate multimodal multiscale data as well as to enable reproducibility of complex experiments. One example of such experimentation is spatially resolved plant root studies, which are difficult to standardize in the field and to conduct under simplistic conditions in the laboratory.

For that purpose, EcoPODs are highly controlled climate chambers that bridge reproducible laboratory and complex field experimental platforms. Equipped with above- and below ground sensors, it allows multimodal data acquisition and monitoring of plants through their entire life cycles. Using the EcoPOD, we conducted a drought experiment with a perennial biofuel crop and collected data on the root architecture and chemical composition from multiple specimens [9]. In order to do so, we stained complete plant root systems for lignin and cellulose. 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 [4].

Cellulose and lignin contents in plant roots have been of interest because they can have direct impact on plant development and health [1], soil erosion prevention [2], and because they can determine the rate of plant decay and hence carbon sequestration rates [3]. Cellulose and lignin concentrations changes in roots have been found to be correlated to agricultural management techniques [5] and microbiome colonization [8]. 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 *P. hallii*. We consider two data sources operating at two different scales: confocal microscopy and X-Ray microtomography. This paper will discuss preliminary results on quantifying phenotypical and chemical variations from multiscale imaging of *P. hallii* for: (a) accessing lignin and cellulose content by visualizing colocalization from images acquired with a laser scanning confocal microscope, followed by (b) calculating variation of chemical profiles across samples. We also illustrate the relationship between lignin and cellulose across root tips.

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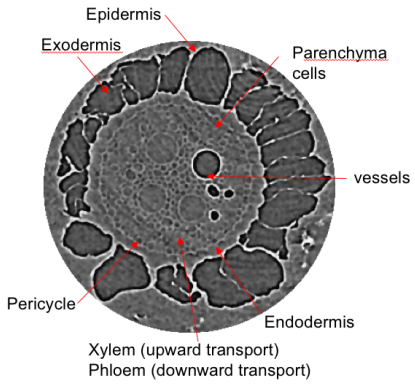

Fig. 1: Transverse cut of *P. hallii*: anatomical plant structures from microCT image slice.

II. Materials Methods

P. 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, since it presents a tractable diploid alternative study system to the tetra- or octaploid biofuel crop switchgrass (Panicum virgatum) [6].

This plant variety stems from the mesic parts of Texas, exhibits delayed flowering and produces 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 analysis of root hair density and surface area, as well as cellulose-lignin ratios along different root segments as a function of drought stress.

P. hallii seeds underwent vapor-phase sterilization as described in [7]. Seedlings were transferred to agricultural soil from the University of California, Davis, and cultivated at 26^oC atmospheric temperature with 12 hour day-night cycles. 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 X-ray CT, and for confocal imaging, as described in the subsections.

Sample preparation for X-ray CT: 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.

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. Resolution is 2.4089 pixels per micron, with voxel size of 0.4151x0.4151x1 micron^3.

III. Preliminary Results

We analyzed two specimens (Figure 2) 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. 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. 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 a half of the cellulose expression for the specimen under drought in comparison with control.

Our results provide baseline data that demonstrate the applicability of multimodal imaging on root of Panicum 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.

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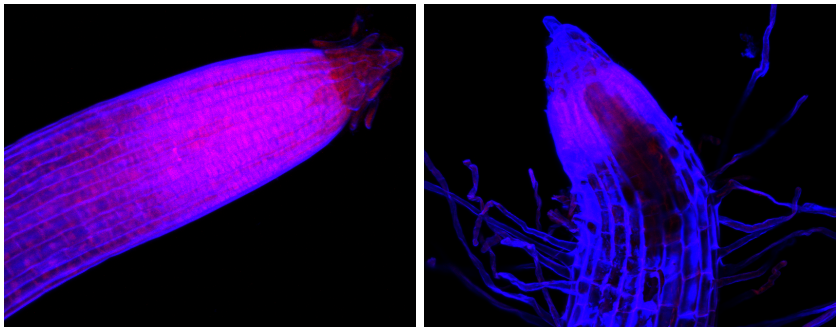

Fig. 2: Longitudinal view of control (left) and drought-regime (right) specimens of P. hallii, both images showing lignin stained with basic fuchsin shown in red tones and cellulose stained with calcofluor white shown in blue tones.

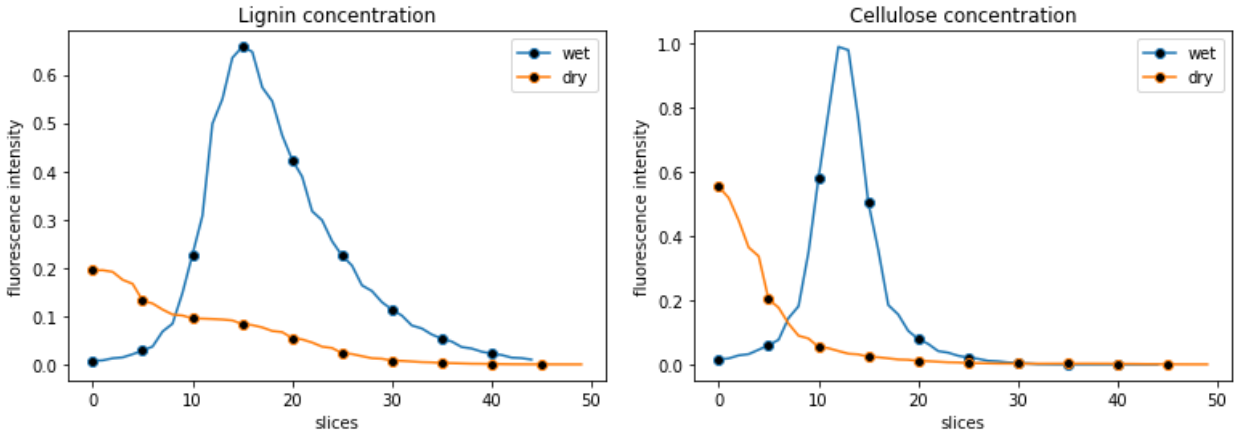

Fig. 3: Longitudinal view of P. hallii control specimen: lignin (left) and cellulose (right) revealing maxima of fluorescence intensity at the root tip.

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