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Submitted

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Hazards:

Profiling Arabidopsis mutants for epicuticular waxes composition towards drought tolerance using infrared spectroscopy

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Subject Areas: Earth & Environmental Sciences

Keywords: Arabidopsis, drought, epicuticular waxes, infrared spectroscopy, multivariate analysis

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Abstract

A growing body of information clearly indicates that significant differences in plant resistance/susceptibility to drought, pathogens, and insects are a function of wax accumulation (load) and wax composition, both of which vary among plant species. However, the relationship between the wax load and composition, wax structure/architecture in the cuticle and tolerance to environmental stresses is still poorly understood. This knowledge is critical for increasing crop tolerance to both abiotic and biotic stresses. It has been shown that Arabidopsis plants subjected to drought or salinity treatments increased the amount of epicuticular wax (Kosma et al. 2009). The goal of this project is to evaluate the potential of mid infrared spectroscopy and spectromicroscopy as a non-destructive and rapid method for high throughput wax-phenotyping to distinguish drought tolerance using Arabidopsis wax mutants as a model system. The mid infrared spectromicroscopy method will elucidate the wax composition and structure-function correlation. Mapping the distribution of wax in the epicuticular and intracuticular layers at high spatial resolution will be helpful to correlate with drought tolerance. Furthermore, this proposed work will further our understanding of the genetic basis of wax biosynthetic and biomechanical properties of leaf cuticular waxes.

Scientific Description

1) What is to be studied? This project is to evaluate the potential of mid infrared spectroscopy and spectromicroscopy as a non-destructive and rapid method for high throughput wax-phenotyping to distinguish drought tolerance using Arabidopsis wax mutants as a model system. The cuticle, in addition to other roles in plant abiotic or -biotic interactions, is the primary barrier for non-stomatal transpirational water loss. The cuticle in the Arabidopsis leaf is composed of epicuticular wax crystals, epicuticular wax film, and intracuticular/intracellular wax or cutin. The cutin layer is physically associated with the epidermal cell wall polysaccharides [1]. Therefore, the cuticle layer in Arabidopsis can be divided into two major layers: Cuticle proper (< 0.25 micron thick) which is composed of the epicuticular wax film, and Cuticle layer (~ 0.5 micron thick) composed of intracuticular wax, cutin and the epidermal cell wall polysaccharides [1]. Epicuticular wax is responsible for the glossy appearance of leaves. The leaf cuticular wax is mainly composed of long chain fatty acids, alkanes, aldehydes, alcohols, and esters. However, epicuticular wax blooms can be deposited on leaf surfaces in different arrangements and are comprised of different chemical compositions depending upon the plant species. Cyclic compounds such as triterpenoids may also occur in the intracuticular wax. Cuticle biosynthesis is a complex phenomenon associated with biotic or abiotic stress response and cuticle composition changes are induced by drought, heat, salinity, and hormone treatments (particularly, ABA). It has been shown that Arabidopsis plants subjected to drought or salinity treatments increased the amount of epicuticular wax [2]. A growing body of information clearly indicates that significant differences in plant resistance/susceptibility to drought, pathogens, and insects are a function of wax accumulation (load) and wax composition, both of which vary among plant species. However, the relationship between the wax load and composition, wax structure/architecture in the cuticle and tolerance to environmental stresses is still poorly understood. This knowledge is critical for increasing crop tolerance to both abiotic and biotic stresses. Therefore, known Arabidopsis wax mutants that differ in cuticular wax compositions will be used here to study the influence of leaf epicuticular waxes in imparting drought tolerance. 2) What experiments will be carried out and what hypothesis will be tested? The goal of the proposed research is to: 1) use Arabidopsis mutants with known alterations in wax load and composition to map the distribution of wax components in the cuticle and correlate this distribution with drought tolerance; 2) identify key wax components that influence drought tolerance; and 3) enhance protective properties of the Arabidopsis cuticle against drought using available wax biosynthetic genes (proof of concept). The hypothesis of study is that leaf wax load and composition of wild type and wax mutants play different roles in tolerance to drought. 3) How will the results impact the field? The mid infrared spectromicroscopy method will elucidate the wax composition and structure-function correlation. Mapping the distribution of wax in the epicuticular and intracuticular layers at high spatial resolution will be helpful to correlate with drought tolerance, as the contribution of epicuticular and intracuticular wax layer to water loss is different. Furthermore, the use of known mutants will enable a proof of concept. This proposed work will further our understanding of the genetic basis of wax biosynthetic and biomechanical properties of leaf cuticular waxes. Once the key wax components that influence drought tolerance are identified, we will create gain-of-function transgenic Arabidopsis lines expressing relevant wax biosynthetic genes in an epidermis-specific manner. We will then determine their leaf wax load and composition by gas chromatography. Lines exhibiting substantially increased wax load and those with wax compositions of interest will be spectroscopically phenotyped and evaluated for enhanced drought tolerance. The knowledge from this study will be delivered and expanded to more crops growing under drought stress. 4) What is the likelihood of success? All members in the research team have extensive experience in synchrotron application, Mid-IR spectroscopy, plant imaging and data analysis. The main applicant has just successfully completed a project on using ATR-FTIR (a Mid-IR technique) to study the effects of genotype and heat stress on the composition of leaf epicuticular wax of filed pea. Mature analytical scenario of spectral profiling collected from plant materials, especially for leaf cuticle, has been developed from the applicant's previous work. For example, characteristic peaks, such as one peak in the methyl CH_3 region (2975-2945 cm-1), and two peaks in the methylene CH_2 region (2945-2820 cm-1), including the CH_2 asymmetric band (2945-2866 cm-1) and the CH_2

symmetric band (2866-2820 cm-1), will be focused to compare the performance of wax mutants grown under drought conditions. References 1. Yeats TH, Rose JKC: The Formation and Function of Plant Cuticles. Plant Physiology 2013, 163(1):5-20. 2. Kosma DK, Bourdenx B, Bernard A, Parsons EP, L S, Joubs J, Jenks MA: The Impact of Water Deficiency on Leaf Cuticle Lipids of Arabidopsis. Plant Physiology 2009, 151(4):1918-1929.

Capability & Productivity of Research Team

Dr. Na Liu is now working as Research Associate of Plant Phenotyping and Imaging Research Centre (P2IRC), to carry out Arabidopsis Chemotyping project (funded by the Global Institute for Food Security [GIFS]) in the Canadian Light Source. Dr. Na Liu has 1.5 years of postdoctoral experience (Department of Plant Sciences, University of Saskatchewan) in studying the role of leaf epicuticular wax in heat tolerance in crops (field pea). She designed experiments to collect data from 11 cultivars and 3 environments, and developed several protocols of Mid-IR spectroscopic work and chemical analysis. She also had experience in growing plants in phytotron and greenhouse. Based on the results of this study, a manuscript (title: Characterizing genotypic and heat stress effects on leaf cuticles of field pea using ATR-FTIR spectroscopy) has been submitted to Frontiers in Physiology-Plant Physiology (IF= 4.13). Another two manuscripts are under preparation. In addition, Dr. Na Liu has 6 years of experience in carrying out spot measurement, bulk measurement and bio-imaging/mapping using diverse spectroscopy combined with conventional and synchrotron-based light source, at national research facilities in Canada, USA and Denmark. She also has 9 years of experience in applying multivariate analysis (chemometrics) to spectral analysis, multi-compounds profiling and multivariate statistical analysis, with certificates from world's top universities. Her previous synchrotron experience came from National Synchrotron Light Source (NSLS) in Brookhaven National Laboratory (USA), where she conducted spot sampling and mapping using synchrotron-based FTIR microspectroscopy to study the inherent structure of hull, seed and endosperm of barley. She, as the first author, has published 9 scientific papers in leading peer-reviewed journals, four publications of which were from her globar- and synchrotron-based Mid-IR spectroscopic work, as listed below: Na Liu, Peiqiang Yu (2016) Recent Research and Progress in Food, Feed and Nutrition with Advanced Synchrotron-Based SR-IMS and DRIFT Molecular Spectroscopy. Critical Reviews in Food Science and Nutrition, 56 (6): 910-918. Na Liu, Peiqiang Yu (2011) Molecular Clustering, Interrelationships and Carbohydrate Conformation in Hull and Seeds among Barley Cultivars. Journal of Cereal Science, 53 (3): 379-383. Na Liu, Peiqiang Yu (2010) Characterization of the Microchemical Structure of Seed Endosperm within a Cellular Dimension among Six Barley Varieties with Distinct Degradation Kinetics, Using Ultraspatially Resolved Synchrotron-Based Infrared Microspectroscopy. Journal of Agricultural and Food Chemistry, 58 (13): 7801-7810. Na Liu, Peiqiang Yu (2010) Using DRIFT Molecular Spectroscopy with Uni- and Multivariate Spectral Techniques to Detect Protein Molecular Structure Differences among Different Genotypes of Barley. Journal of Agricultural and Food Chemistry, 58 (10): 6264-6269. Dr. Chithra Karunakaran is staff scientist at the Canadian Light Source with specialization in infrared, soft, and hard X-ray spectromicroscopic data. She is the senior group leader of plant imaging. Mr. Jarvis is now working as support scientist in plant imaging at the Canadian Light Source. He has extensive experience in infrared, x-ray spectroscopy and imaging analysis.

Societal, Economic and Industrial Relevance

SOCIETAL AND ECONOMIC IMPACT:

This study aims to use cutting-edge synchrotron-based Mid-IR techniques to study the effects of drought on the model plant, Arabidopsis thaliana. The results from this study will provide a solid knowledge foundation for biochemical composition of leaf cuticles, enabling Canadian researchers to develop and test new concepts prior to their applications to other crops. The powerful synchrotron-based imaging technologies will provide high-quality in-situ phenotypic characterization of plants, make it possible to further develop high-throughput and non-destructive methods to screen extensive genomic resources for sustainable agriculture.

INDUSTRIAL RELEVANCE:
The results from this study will help to develop novel high-throughput screening method for superior genotypes of crops.

Materials & Methods

MID-IR – Mid-Infrared Spectromicroscopy

8 Shifts

Suitability and Justification:

The third generation synchrotron in the CLS is of crucial importance for this study, as we are going to take the advantage of the high brightness and high signal to noise ratio from the synchrotron source to investigate the leaf cuticles at cellular and sub-cellular level. The mid infrared spectroscopy is a vibrational spectroscopy method with distinct features for different biomolecules like proteins, lipids, and cell wall polysaccharides. Further, the spectra are sensitive to the chemical bonds and functional groups within a biomolecule (e.g. saturated vs unsaturated lipids; alkanes vs alcohol). Although infrared spectroscopy and microscopy are available based on a Globar source (lab based), the synchrotron source has high spatial resolution and signal-to-noise ratio. Therefore, the mid infrared spectromicroscopy method will elucidate the wax composition and structure-function correlation. Mapping the distribution of wax in the epicuticular and intracuticular layers at high spatial resolution will be helpful to correlate with drought tolerance, as the contribution of epicuticular and intracuticular wax layer to water loss is different. Furthermore, the use of known mutants will enable a proof of concept. This proposed work will further our understanding of the genetic basis of wax biosynthetic and biomechanical properties of leaf cuticular waxes.

Source	Bending Magnet
Spectral Range	560 - 6000 cm^-1
Resolution	16.0 – 0.125 cm^-1
Spot Sizes	Diffraction Limited
Photon Flux	10^14 @ 10 μm

Mid-Infrared Spectromicroscopy (MID-IR)

The Mid Infrared Spectromicroscopy beamline provides a state-of-the-art Fourier Transform IR spectrometer and microscope to supply diffraction-limited spatial resolution to an ever-widening range of infrared spectroscopy experiments. Research and development will explore new experiments and re-examine existing techniques by applying the advantages of high brightness infrared synchrotron light.
http://midir.lightsource.ca/

Experimental Procedure:

Fourier Transform Infrared Spectroscopy (FTIR)

1) Plant materials: The wild type and five Arabidopsis mutants, cer1-4, cer2-5, cer3-6, cer4-4, and cer6, will be used in our study. Arabidopsis seeds will be seeded in soil (Sunshine Mix 4, SunGro) in 5-inch pots at a density of six plants per pot. Plants will be grown at 20 degrees celsius with a 16/8-h light/dark cycle and a light

intensity of 100-120 micro mol m-2 s-1 (photosynthetically active radiation). Fifteen to sixteen-day-old plants (rosette stage) will be used for drought treatment. Plants will be deprived of water until wilting of lower leaves is observed (typically 7-9 d after start of treatment). Rosette leaves from water-deprived plants and from untreated control plants will be harvested at the same time for Mid-IR spectroscopic work. 2) Mid-IR Spectroscopy: The epicuticular wax thickness and composition of wild type and mutant lines (control and drought stressed) will be evaluated using the surface sensitive, attenuated total internal reflection (ATR) mid infrared spectroscopy method (the offline FTIR instrument in the Canadian Light Source will be used for this experiments). This method measures the wax components from the adaxial and abaxial sides of freshly collected leaves and 2-3 spectra will be collected per leaf samples to reduce the variability on a leaf surface. Selected leaf samples will be cut into thin cross-sections (around 8 micron thickness) using a cryo-microtome for high-resolution mid-IR mapping at the mid infrared beamline (01B1-1) at the Canadian Light Source. The synchrotron beam at a spatial resolution up to 1 micron using oversampling method, combined with a FTIR microspectroscopy, will be used for data collection. Beamtime: 4 beamtime shifts in Cycle 27 will be requested to collect high-quality Mid-IR imaging of cross-sections of Arabidopsis leaves, consisting of samples from wild type and 2 mutants: wild type + cer 1-4 + cer 2-5, 2 treatments/mutant(type), and 2 repeats/combination. Based on the results from the first 4 shifts, the procedures of data collection and analysis will be optimized and another 4 beamtime shifts in Cycle 28 will be requested to collect high-quality Mid-IR imaging of the samples from the rest four mutants. 3) Data analysis: Mature analytical scenario of spectral profiling collected from plant materials has been developed from Dr. Na Liu's previous work. Uni- and multivariate data analysis will be used to analyze the Mid-IR spectromicroscopic data and investigate differences in cuticle composition among the mutants under control and drought conditions. Agglomerative hierarchical cluster analysis (CLA) and principal component analysis (PCA) will be applied to visualize the differences of spectroscopic characteristics. The two multivariate analyses will be performed using the PLS-toolbox software (Eigenvector Research, Inc., WA, USA). 4) Role of research team members: Dr. Na Liu will design experiment plan, prepare samples, perform experiments, and analyze data. Mr. Jarvis Stobbs will provide support and assistance to Dr. Na Liu for data collection. Dr. Chithra Karunakaran will supervise the project.

The material below is the one which was submitted with the original proposal. The project which was created from this proposal has since has its material amended.

Please see the project detail page at 27G09064~Liu if you wish to see up-to-date information for the project.

Name	Description	Type	Quantity	Hazards
Arabidopsis (1) (6 * 24 leaves)	Treatment: no	bio_other	6 * 24 leaves	☑
Arabidopsis (2) (6 * 24 leaves)	Treatment: drought	bio_other	6 * 24 leaves	☑

Sample Preparation:

BIOLOGICAL MATERIALS SAMPLES

Biological Materials(BM) Involved: Y

NANOMATERIALS

NM-How are the samples contained a substrate:

Waste Generation:

No waste will be generated.

Appendix: Attachments

File	Type	Owner	Size	Added
27-9064 Liu (Mid IR).pdf	Scientific	Na Liu	42.3 KB	2018-01-08 18:29