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Submitted

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Hazards:

Multi-elemental profiling of Arabidopsis mutants towards drought tolerance

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Subject Areas: Agricultural, Veterinary & Food Sciences, Earth & Environmental Sciences

Keywords: Arabidopsis, drought, elemental profiling, XRF, multivariate analysis

Funding Sources: Global Institute for Food Security,

Abstract

This proposed study is the Part II of the Arabidopsis Chemotyping Project (funded by the Global Institute for Food Security [GIFS]) in the Canadian Light Source. Nutrients uptake in plant tissues is a complex phenomenon associated with genetic and environmental factors. The elemental concentration and distribution may exhibit changes or modification in response to biotic or abiotic stresses. The proposed study will study the effects of genotype and drought stress on the elemental composition of leaf and root tissues of Arabidopsis mutants using Synchrotron-based X-ray fluorescence spectroscopy (XRF). The objectives of this study are: 1) to determine the multi-elemental concentrations in the leaves and roots of Arabidopsis using synchrotron-based XRF technique; 2) to visualize the in situ distribution of multi-elements in the leaves and roots of Arabidopsis mutants at cellular and sub-cellular level; 3) to identify key chemical elements that influence drought tolerance and correlate their distribution to evaluate the interaction between multiple elements in the Arabidopsis mutants grown under two regimes. The results from this experiment will be combined with the findings from Part I of the Arabidopsis Chemotyping Project in order to gain a deeper understanding of how plants respond to drought stress.

Scientific Description

This proposed study is the Part II of the Arabidopsis Chemotyping Project (funded by the Global Institute for Food Security [GIFS]) in the Canadian Light Source, under the collaboration within the Plant Phenotyping and Imaging Research Centre (P2IRC). The Part I of the GIFS Arabidopsis Chemotyping Project on using ATR-FTIR (a Mid-IR technique), Profiling Arabidopsis mutants for epicuticular waxes composition towards drought tolerance using infrared spectroscopy, has been accomplished at the Mid-IR beamline of the Canadian Light Source.

Nutrients uptake in plant tissues is a complex phenomenon associated with genetic and environmental factors. The elemental concentration and distribution may exhibit changes or modification in response to biotic or abiotic stresses, such as pathogen drought, heat, salinity, and hormone treatments (particularly, ABA) (Ghandilyan et al. 2009). It has been shown that plants subjected to bacteria treatments reduced Zn concentration on their leaves (Tian et al. 2014).

A growing body of information clearly indicates that significant differences in plant resistance/susceptibility to environmental stress are a function of diverse chemical composition, which varies among plant species (Urano et al. 2010). However, the relationship between the elemental composition 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 mutants will be used here to study the elemental composition in imparting drought tolerance, in order to obtain a better understanding of the regulation of elements in plant tissues and improve screening approaches in plant breeding.

Synchrotron-based X-ray fluorescence spectroscopy (XRF) is a powerful tool to conduct multi-elemental imaging at high spatial resolution (Kopittke et al. 2018; Punshon et al. 2013). The proposed study will evaluate the potential of XRF as a rapid method for high-throughput phenotyping to distinguish drought tolerance using Arabidopsis mutants as a model system. Mapping the distribution of elements in the leaves and roots of Arabidopsis will also be helpful to correlate multi-elemental profiles with plant drought tolerance.

The hypothesis of study is that elemental composition in leaf and root tissues of wild type and mutants significantly differ in response to drought. The objectives of this study are: 1) to determine the multi-elemental concentrations in the leaves and roots of Arabidopsis using synchrotron-based XRF technique; 2) to visualize the in situ distribution of multi-elements in the leaves and roots of Arabidopsis mutants at cellular and sub-cellular level; 3) to identify key chemical elements that influence drought tolerance and correlate their distribution to evaluate the interaction between multiple elements in the Arabidopsis mutants grown under two regimes.

The likelihood of success: all members in the research team have extensive experience in synchrotron application, spectroscopy, plant imaging and data analysis. The main applicant has just successfully completed the Part I of the Arabidopsis Chemotyping Project on using ATR-FTIR (a Mid-IR technique) to study the effects of genotype and drought stress on the composition of leaf epicuticular wax of Arabidopsis.

A pilot experiment of multi-elemental profiling of Arabidopsis leaves with a 0.5 mm resolution has been done in the Industry, Development, Education, Applications, Students (IDEAS) beamline at the Canadian Light Source. The bulk analysis of element composition showed that cer2-5 cer2like1-1 mutant grown under the two regimes accumulated more Ca but less K than the wild type in leaves (Fig 1 attached). Mature analytical scenario of multi-elemental profiling on plant materials has been developed from this pilot work.

References:

Ghandilyan, A., Barboza, L., Tisne, S., Granier, C., Reymond, M., Koornneef, M., . . . Aarts, M. G. M. (2009). Genetic analysis identifies quantitative trait loci controlling rosette mineral concentrations in Arabidopsis thaliana under drought. New Phytologist, 184(1), 180-192.

Kopittke, P. M., Punshon, T., Paterson, D. J., Tappero, R. V., Wang, P., Blamey, F. P. C., . . . Lombi, E. (2018). Synchrotron-Based X-Ray Fluorescence Microscopy as a Technique for Imaging of Elements in Plants. Plant Physiology, 178(2), 507-523.

Punshon, T., Ricachenevsky, F. K., Hindt, M. N., Socha, A. L., & Zuber, H. (2013). Methodological approaches for using synchrotron X-ray fluorescence (SXRF) imaging as a tool in ionomics: examples from Arabidopsis thaliana. Metallomics, 5(9), 1133-1145.

Sole, V. A., Papillon, E., Cotte, M., Walter, P., & Susini, J. (2007). A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra. Spectrochimica Acta Part B-Atomic Spectroscopy, 62(1), 63-68.

Tian, S. K., Lu, L. L., Labavitch, J. M., Webb, S. M., Yang, X. E., Brown, P. H., & He, Z. L. (2014). Spatial imaging of Zn and other elements in Huanglongbing-affected grapefruit by synchrotron-based micro X-ray fluorescence investigation. Journal of Experimental Botany, 65(4), 953-964.

Urano, K., Kurihara, Y., Seki, M., & Shinozaki, K. (2010). 'Omics' analyses of regulatory networks in plant abiotic stress responses. Current Opinion in Plant Biology, 13(2), 132-138.

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. She has accomplished the Part I of this project: Profiling Arabidopsis mutants for epicuticular waxes composition towards drought tolerance using infrared spectroscopy. Based on her results, a manuscript (title: Mid-infrared spectroscopy is a fast screening method to select Arabidopsis genotypes based on leaf epi-cuticular wax) has been prepared and under revision.

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. Her previous synchrotron experience came from National Synchrotron Light Source (NSLS) in Brookhaven National Laboratory (USA) and the Canadian Light Source. She, as the first author, has published 9 scientific papers in leading peer-reviewed journals, five publications of which were from her globar- and synchrotron-based Mid-IR spectroscopic work. Dr. Na Liu’s PhD project was on elemental profiling of biomass, based on which four scientific papers have been published in international peer-reviewed journals. 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. Dr. Na Liu’s publications on abiotic stress on plants, synchrotron-based spectroscopy, elemental profiling and multivariate analysis are listed below:

Liu N., Karunakaran C., Lahlali R., Warkentin T. & Bueckert R.A. (2019) Genotypic and heat stress effects on leaf cuticles of field pea using ATR-FTIR spectroscopy. Planta, 249, 601-613.
Liu N., Larsen S.U., Jorgensen U., Murach D., Pflugmacher C., Hartmann H. & Laerke P.E. (2017) Combustion quality of poplar and willow clones grown as SRC at four sites in Brandenburg, Germany. Biomass & Bioenergy, 106, 51-62.
Liu N., Yu P. (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.
Liu N., Yu P. (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.

Dr. Chithra Karunakaran is Manager- Environmental and Earth Sciences at the Canadian Light Source with specialization in infrared, soft, and hard X-ray spectromicroscopy. She is the senior group leader of plant imaging at the Canadian Light Source.

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

This study aims to use cutting-edge synchrotron-based X-ray techniques to study the effects of drought on the model plant, Arabidopsis thaliana, at micron scale. The results from this study will provide a solid knowledge foundation for elemental composition, transportation and distribution in leaf and root tissues, 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, making it possible to further develop high-throughput and non-destructive methods to screen extensive genomic resources for sustainable agriculture.

Materials & Methods

Consider for other beamlines
The spokesperson of this proposal has indicated that they would like to be considered for other appropriate beamlines.

VESPERS – Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron

6 Shifts

Suitability and Justification:

Traditional wet chemical analysis loses the special-resolved information of the intact plant tissues. The third generation synchrotron in the CLS is thus of crucial importance for this study. The research team is going to take the advantage of the high brightness and high signal to noise ratio from the synchrotron source to accomplish the multi-element mapping of the leaf and root tissues of Arabidopsis with micron resolution. Furthermore, the use of known mutants will enable a proof of concept of using the synchrotron-based XRF techniques at the VESPERS beamline to better understand the nutrients transport and distribution in the leaves and roots. This proposed work will expand our

Source	Bending Magnet
Spectral Range	6 – 30 keV
Resolution	Si-111: 10^{-4}, MLM1: 10^{-2} , MLM2: 10^{-1}, Polychromatic Beam (Pink Beam)
Spot Sizes	2 µm to 4 µm (mm-sized beam also available)
Photon Flux	Si-111: $2x10^{9}$, MLM1: $1x10^{11}$, MLM2: $4x10^{11}$ (@15 keV and 100 mA)

Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron (VESPERS)

VESPERS is a hard X-ray microprobe beamline capable of providing a high level of complementary structural and analytical information. The techniques of X-ray diffraction and X-ray fluorescence spectroscopy are employed to analyze a understanding on how plant regulates nutrient uptake and translocation at cellular and sub-cellular level.

microscopic volume in the sample, as well as X-ray absorption spectroscopy. Multi-bandpass and pink beam capability are built in to meet variable requirements. http://vespers.lightsource.ca/

Experimental Procedure:

Micro XAFS/XRD/XRF Imaging **X-ray Fluorescence Spectroscopy (XRF)**

1) Plant materials: The wild type and five Arabidopsis mutants, cer1-4, cer2-5 cer2like1-1, 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). Two-week-old plants (rosette stage) will be randomly separated for control and drought treatment. Plants in drought group will be deprived of water until wilting of lower leaves is observed (2 weeks after start of treatment). Plants in control group will still be watered as normal. Water-deprived plants and control plants will be harvested at the same time for XRF work. The plants will be flash frozen by liquid nitrogen to preserve the cellular structure. For each genotype-treatment combination, three replicates will be used.

2) XRF Spectroscopy: The leaves and roots tissues of wild type and mutant lines (control and drought stressed) will be cryo-sectioned to 80 µm sections in a Leica CM1950 cryostat microtome (Leica Biosystems Inc., Canada). The entire leaves and the cross-sections of leaves and roots will be evaluated using the X-ray Fluorescence spectroscopy methods at the Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron (VESPERS) beamline in the Canadian Light Source. The XRF method will be used to measure the concentrations and distributions of K, Ca, Mn, Fe, Ni, Cu and Zn in the Arabidopsis leaves and roots. XRF calibration standards with known concentrations of the target elements will be measured under the same conditions for the elemental concentrations in the plant samples.

3) Beamtime: The 6 genotypes grown under 2 regimes with sampling from leaves and roots makes 24 samples in total. The first beamtime shift will be requested to collect elemental profiling data of the wild type. Based on the results from the first shift, the procedures of data collection and analysis will be optimized and the rest 5 beamtime shifts will be requested to collect data from the five mutants. Because of the capacity of the growth chamber, the plant growing plan is designed as one genotype to be harvest per week. Therefore, the samples will come every week for 6 weeks.

4) Data analysis: The data obtained from VESPERS will be processed using the PyMCA software package (Solé et al. 2007). Mature analytical scenario of multi-elemental profiling collected from plant materials has been developed from Dr. Na Liu's previous work at the IDEAS beamline in the Canadian Light Source. In addition, uni- and multivariate data analysis will be used to analyze the bulk XRF data and investigate differences in elemental profiling 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 elemental profiles.

5) 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.

CLS-APS – CLS APS ACCESS – Sector 20 Beamlines

6 Shifts

Suitability and Justification:

Traditional wet chemical analysis loses the special-resolved information of the intact plant tissues. The synchrotron light source is thus of crucial importance for this study. The research team is going to take the advantage of the high brightness, resolution and signal to noise ratio from the synchrotron source to accomplish the multi-element mapping of the leaf and root tissues of Arabidopsis with micron resolution. Furthermore, the use of known mutants will enable a proof of concept of using the synchrotron-based XRF techniques to better understand the nutrients transport and distribution in the leaves and roots. This proposed work will expand our understanding on how plant regulates nutrient uptake and translocation at cellular and sub-cellular level.

Source	Bending Magnet, Insertion Device
Spectral Range	ID: 4.3 – 27 keV(Si111), 8 – 50 keV (Si311); BM: 2.7 – 32.7 keV
Resolution	Si(311) 0.3×10^{-4}, Si(111) 1.4×10^{-4}
Spot Sizes	2μm, 5μm, 400μm x 100μm
Photon Flux	10^{11} (BM), 10^{13}, 2×10^{12}(ID)

CLS APS ACCESS – Sector 20 Beamlines (CLS-APS)

CLS APS ACCESS – Sector 20 Beamlines
http://s20.xray.aps.anl.gov/beamlines.html

Experimental Procedure:

Macro XAFS/XRF Imaging **Micro XAFS/XRF Imaging** **X-ray Fluorescence Spectroscopy (XRF)**

1) Plant materials: The wild type and five Arabidopsis mutants, cer1-4, cer2-5 cer2like1-1, 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). Two-week-old plants (rosette stage) will be randomly separated for control and drought treatment. Plants in drought group will be deprived of water until wilting of lower leaves is observed (2 weeks after start of treatment). Plants in control group will still be watered as normal. Water-deprived plants and control plants will be harvested at the same time for XRF work. The plants will be flash frozen by liquid nitrogen to preserve the cellular structure. For each genotype-treatment combination, three replicates will be used.

2) XRF Spectroscopy: The leaves and roots tissues of wild type and mutant lines (control and drought stressed) will be cryo-sectioned to 80 μm sections in a Leica CM1950 cryostat microtome (Leica Biosystems Inc., Canada). The entire leaves and the cross-sections of leaves and roots will be evaluated using the X-ray Fluorescence spectroscopy methods. The XRF method will be used to measure the concentrations and distributions of K, Ca, Mn, Fe, Ni, Cu and Zn in the Arabidopsis leaves and roots. XRF calibration standards with known concentrations of the target elements will be measured under the same conditions for the elemental concentrations in the plant samples.

3) Beamtime: The 6 genotypes grown under 2 regimes with sampling from leaves and roots makes 24 samples in total. The first beamtime shift will be requested to collect elemental profiling data of the wild type. Based on the results from the first shift, the procedures of data collection and analysis will be optimized and the rest 5 beamtime shifts will be requested to collect data from the five mutants. Because of the capacity of the growth chamber, the plant growing plan is designed as one genotype to be harvest per week. Therefore, the samples will come every week for 6 weeks.

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analysis will be used to analyze the bulk XRF data and investigate differences in elemental profiling 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 elemental profiles.

5) 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.

Name	Description	Type	Quantity	Hazards
Arabidopsis (6 genotypes * 2 treatments (Control and Drought))	4-week-old plants	plant	6 genotypes * 2 treatments (Control and Drought)	☑

Sample Preparation:

1) Plant materials: The wild type and five Arabidopsis mutants, cer1-4, cer2-5 cer2like1-1, 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). Two-week-old plants (rosette stage) will be randomly separated for control and drought treatment. Plants in drought group will be deprived of water until wilting of lower leaves is observed (2 weeks after start of treatment). Plants in control group will still be watered as normal. Water-deprived plants and control plants will be harvested at the same time for XRF work. The plants will be flash frozen by liquid nitrogen to preserve the cellular structure. For each genotype-treatment combination, three replicates will be used.

2) XRF Spectroscopy: The leaves and roots tissues of wild type and mutant lines (control and drought stressed) will be cryo-sectioned to 80 µm sections in a Leica CM1950 cryostat microtome (Leica Biosystems Inc., Canada). The entire leaves and cross-sections will be evaluated using the X-ray Fluorescence spectroscopy methods at VESPERS and/or CLS@APS beamlines in the Canadian Light Source. The XRF method will be used to measure the concentrations and distributions of K, Ca, Mn, Fe, Ni, Cu and Zn in the Arabidopsis leaves and roots. XRF calibration standards with known concentrations of the target elements will be measured under the same conditions for the elemental concentrations in the plant samples.

Waste Generation:

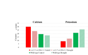
The following types of waste will be generated:

- Non-Hazardous Waste

Waste Disposal:

The cross-sections of Arabidopsis plants is non-hazardous.

Appendix: Attachments

File	Type	Owner	Size	Added	
Pilot results	Scientific	Na Liu	44.4 KB	2019-08-21 12:40	

Pilot results [Scientific]

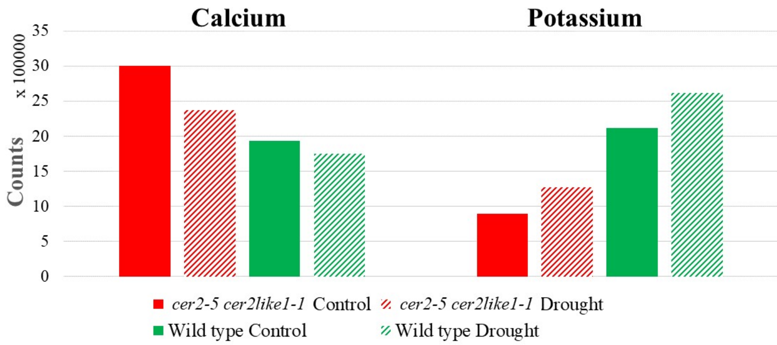


Fig 1. Counts differences of calcium and potassium in the rosette leaves of 4-week-old Arabidopsis plants, in terms of synchrotron-based X-ray fluorescence spectroscopy signals: comparing between the mutant *cer2-5 cer2like1-1* and the wild type grown under Control and Drought regimes. This pilot experiment was done in the Industry, Development, Education, Applications, Students (IDEAS) beamline at the Canadian Light Source.