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Submitted

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Hazards:

Crown freezing behaviors and avenues for future research on tissue-specific physiological and biochemical markers targeted to tissue-specific freezing survival in wheat varieties

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Abstract

Despite a 30 % higher yield than conventional spring wheat crops, overwintering survival limits winter wheat to acreages in the Southern areas in Alberta and Saskatchewan. Mitigation and control of ice formation within the critical crown organ is paramount for winter wheat regrowth and subsequent grain yields. The accumulation of large ice crystals in the crown injures meristem cells required for spring root and shoot regrowth. Exposure of winter rye to non-freezing, cold-acclimating temperatures, lowers water concentrations in crown vascular tissue and a putative barrier region between the vascular transition zone and the crown shoot apical meristem as compared with winter wheat (Willick et al. 2019, Plant Cell & Environment 42: 701-716). However, little is known about the morphology of this barrier and its role in inhibiting ice propagation. Synchrotron-based X-ray Phase Contrast Imaging and 3D rendering provide a non-destructive technique with the required resolution to characterize winter wheat and rye tissue-specific differences in crown anatomy. This research will expand on previous research (Brar et al. 2019) to standardize protocols for 3D PC imaging of non-frozen and freeze-injured crowns. Second, we will identify tissue-specific structures in rye crowns less susceptible to ice formation injury as compared with winter wheat.

Scientific Description

The purpose of this study is to build on this earlier hypothesis to characterize the properties of the crown intermediate zone. We hypothesize that morphological differences in the intermediate zone increase the freezing survival of winter rye meristem to a greater degree than in wheat. To test this theory, winter wheat cv. Norstar and winter rye cv. Puma will be used as model systems because of their extensive use as traditional check cultivars in overwinter freezing survival studies. Preliminary research from 2016 on wheat crowns and recently published work (Brar et al. 2019 Plant Cell & Environment 42: 509-526) conducted at the Canadian Light Source will form the basis to create higher-resolution PCI 3D models of non-injured and freeze-injured wheat and rye crowns. The objectives of this research are to I) characterize the size and volume of abscesses in the vascular tissue created by ice propagation in winter wheat and the more freezing resistant winter rye. II) Phenotype morphological differences in the intermediate zone between winter wheat and winter rye.

We propose to expand on our proof-of-concept work into a larger project for concrete development and results. As such, this research will be combined with experiments using NMR microimaging to map tissue-specific water accumulation in non-frozen and frozen crowns, traditional histological imaging and wet lab techniques to characterize tissue-specific cell wall compositional changes in response to cold-acclimation and freezing.

Capability & Productivity of Research Team

Dr. Chithra Karunakaran, Ph.D., P. Eng., (CIM) (Staff Scientist at the Canadian Light Source, Adjunct Professor in Chemical and Biological Engineering, University of Saskatchewan). Dr. Karunakarn is a professional Biosystems Engineer and has been working as a Staff Scientist at the CLS since 2005. Presently, she is leading the Plant Innovation Research program at the Canadian Light Source, a unique research project in Canada and the world. Her expertise is conducting agriculture and plant science-related research projects using synchrotron techniques. Her knowledge of synchrotron techniques and her experience in applying to crops and food products will be invaluable to this project. She has published in more than 35 refereed journals, 3 invited book chapters, and over 82 conference proceedings and papers. She is the lead researcher responsible for the successful completion of the one-year ADF project 20130054: Proof-of-concept- Application of Synchrotron Light for Crop Improvement. Dr. Karunakarn will assist as needed in data acquisition and interpretation.

Mr. Stobbs will collect data at the beamline and re-construct the crown 3D models.

Dr. Ian R. Willick, Ph.D. (Post-Doctoral Research Scientist in the Department of Plant Biology, Michigan State University). Dr. Willick worked as a research assistant (2013-2015) at the Canadian Light Source under the supervision of Dr. Karunakaran and participated in projects involving synchrotron FTIR and PCI. He assisted in the successful completion of the ADF project 20130054. Dr. Willick conducted his Ph.D. research (2013-2018) and held a post-doctoral research appointment (2018-2019) with Dr. Karen Tanino characterizing mechanisms of freezing resistance in winter wheat and rye crowns. In the last six years, he has published 10 peer-reviewed papers, 3 invited book chapters and 21 abstracts for conference proceedings. Dr. Willick will contribute off-site in the production of plant material (at the University of Saskatchewan) and the interpretation of results.

Dr. Karen Tanino, Ph.D. (Professor in the College of Agriculture and Bioresources, University of Saskatchewan). Dr. Tanino has taught 14 undergraduate and graduate courses including a biannual graduate course on Plant Abiotic Stress. She has contributed to the field of plant freezing stress for 35 years and co-chaired the 8th International Plant Cold Hardiness Seminar (2007), and served on the Scientific Committee and chaired the pre-conference student course for the 10th (2013 in Poznan, Poland) and 11th (2018 in Madison, Wisconsin) International Plant Cold Hardiness Seminars. Recently, Dr. Tanino has served as the president of the Canadian Society for Horticulture Science (2016-2018) chaired the 2018 Annual General Meeting for the Canadian Society of Horticulture Science (Niagara Falls, Ontario). She has published more than 90 refereed articles

(h-index = 22) and has served as an editor and co-author of five books. Her role in the project will be to direct and provide oversight on the data interpretation and ensure subsequent publications are produced in a timely manner.

Societal, Economic and Industrial Relevance

This research will provide a better understanding of crown freezing behaviours and avenues for future research on tissue-specific physiological and biochemical markers targeted to tissue-specific freezing survival. Further advancements in winter wheat cold hardiness using a tissue-specific approach benefits Western Canadian farmers and a boon to the Canadian agricultural industry.

Materials & Methods

BMIT-BM – Biomedical Imaging and Therapy (BM)

4 Shifts

Suitability and Justification:

A third-generation synchrotron such as CLS can produce X-rays used for a variety of purposes. The previous research by our team members has shown great potential of PCI to better understand the complex internal plant structures and dynamic processes. The synchrotron-based PCI is a minimally invasive technique that can be used successfully to reconstruct internal structures of plants in 3D using micron- to nano-scale resolutions. This overcomes limitations of image quality compared to desktop micro-CT when used to identify small tissue structures such as the putative barrier region. Research by Karunakaran et al. (2015; Scientific Reports 5: 12119) observed the BMIT X-ray beamline has unique properties not possessed by X-ray's generated by conventional micro-CT sources. Synchrotron-based X-rays PCI is superior to traditional sources in terms of flux density, selective wavelengths, and partial coherence. High flux density from synchrotron beams reduces the exposure time and provides a higher signal to noise ratio. This is favourable for studying minute differences in crown tissue anatomy.

Source	Bending Magnet
Spectral Range	12.6 – 40.0 keV
Resolution	<10^-3 mono, ~0.1 filtered white beam
Spot Sizes	200 mm horizontal (flat); 4 mm vertical (FWHM) for mono @ 20 keV and 3 mm vert. (FWHM) for filtered white beam
Photon Flux	10^9 ph/s/mm2 @ 20 keV mono, 10^12 ph/s/mm2 peak @ 20 keV filtered white beam

Biomedical Imaging and Therapy (BM) (BMIT-BM)

The BioMedical Imaging and Therapy (BMIT) Facility is designed for the purpose of imaging biological tissue and conducting radiation therapy research. The BMIT facility will address the interest of scientists and clinicians in the diagnosis and treatment of cancer, circulatory and respiratory disease, neurological and behavioural disease, reproductive dysfunction, musculo-skeletal disease and kinesiology, and dental conditions.
http://bmit.lightsource.ca/

Experimental Procedure:

X-ray Imaging and Microtomography

Fixed winter wheat and winter rye in 90 % ethanol will be imaged in falcon tubes with the Orca Flash 4 utilizing WB microscope 2x mag and 0.9x eyepiece for a pixel-size of 3.61-micron, and subsequent samples of interest images with the 5x magnification for a pixel-size of 1.44 microns.

Ancillary Requirements:

Labs: BMIT Lab, Life Sciences Lab

Name	Description	Type	Quantity	Hazards
Winter Wheat Crown (12)	Wheat crowns fixed in 90 % ethanol.	plant	12	☑
Winter Rye Crowns (12)	Rye crowns fixed in 90 % ethanol.	plant	12	☑

Sample Preparation:

Samples will be brought to site in 15 mL falcon tubes, and subsequently imaged in the same tubes. Samples may need to be placed in another tube and will be done so in the BMIT small animal wearing standard PPE.

Waste Generation:

The following types of waste will be generated:

- Non-Hazardous Waste

Waste Disposal:

Samples are non-hazardous and can be disposed of in regular garbage. Any waster ethanol is be left to evaporate in a fume hood or disposed of in the appropriate waste container.