UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

KARAMJEET SINGH

On Tuesday, September 8, 2020 at 1:30 p.m. (online)

Thesis Title: **Structural and biochemical investigation into *Arabidopsis thaliana* LEA3 proteins**

Examination Committee:

Dr. Annette Nassuth, Molecular and Cellular Biology (Exam Chair)
Dr. Steffen Graether, Dept. of Molecular and Cellular Biology
Dr. Tariq Akhtar, Dept. of Molecular and Cellular Biology
Dr. Ian Tetlow, Dept. of Molecular and Cellular Biology
Dr. Alejandra Covarrubias, Dept. of Plant Molecular Biology, Institute of Biotechnology, Mexico (UNAM) (External Examiner)

Advisory Committee:
Dr. Steffen Graether (Adv)
Dr. Matthew Kimber
Dr. Rod Merrill
Dr. Tariq Akhtar

Abstract: LEA3 proteins are intrinsically disordered proteins expressed in plants during seed development and in response to abiotic stress. Limited studies have shown they can confer stress tolerance in plants, yet their mechanism remains elusive. This thesis aims to further our understanding of the LEA3 group. First, a comprehensive bioinformatics analysis revealed two previously undiscovered C-terminal motifs containing conserved acidic and hydrophobic residues and four N-terminal motifs. Five general architectures were proposed for LEA3 and the physiochemical properties of the different architectures showed clustering in a relatively narrow range compared to the previously studied dehydrins. The evolutionary analysis revealed that the proteins grouped into clades based on their architecture, and that there appears to be at least two distinct groups of LEA3 proteins based on their architectures and physiochemical properties. The presence of LEA3 proteins in non-vascular plants but their absence in algae suggests that LEA3 may have arose in the evolution of land plants. A protocol to express and purify AtLEA3-2 with ^{15}N and ^{13}C isotopes in *E. coli* is described, although the protocol can be adapted for any LEA3 with or without isotopic labeling. The AtLEA 3-2 gene was cloned into the pET-SUMO vector, which allowed for the SUMO-AtLEA 3-2 fusion protein to be purified using Ni-affinity chromatography and, through the use of Ulp1, a SUMO protease, resulted in an AtLEA 3-2 with a native N-terminus. Lastly, several biochemical experiments were performed to elucidate the function of LEA3 proteins. I show that the LEA3 proteins are

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disordered in solution, have regions with propensity for order, and are more hydrophobic than other LEA groups. One member, LEA3-4, bound Cu^{2+} and Fe^{3+} ions with micromolar affinity. All LEA3 proteins were effective cryoprotectants of LDH and showed a gain in α-helicity in the presence of SDS, while only LEA3-4, showed a gain in α-helicity in the presence of the membrane mimic DPC. I used ^{15}N-HSQC NMR to show that the additional W- and DAELR motifs present in LEA3-4 are involved in the interaction with DPC. I conclude that the LEA3 group could have multiple functions in protecting cells during stress.

Curriculum Vitae: Karamjeet received his Bachelor of Science (Honours) Biochemistry at the University of Guelph in April 2016, and then entered directly into the Ph.D. program, in the lab of Dr. Steffen Graether in May 2016.

Awards: Graduate Tuition Scholarship (2016-2020)
Queen Elizabeth II Graduate Scholarship in Science and Technology (2018-2019)

Publications: **Singh, K.K.,** and Graether, S.P. (2020). Expression and Purification of an Intrinsically Disordered Protein in *Intrinsically Disordered Proteins: Methods and Protocols*, Methods in Molecular Biology. Springer Nature.

Singh KK, Graether SP (2020). Conserved sequence motifs in the abiotic stress response protein late embryogenesis abundant 3. PLoS ONE 15(8): e0237177. https://doi.org/10.1371/journal.pone.0237177.

Malik, A., Veltri, M., Boddington K., **Singh, K.,** and Graether, S. (2017). Genome Analysis of Conserved Dehydrin Motifs in Vascular Plants. *Front Plant Sci*. 8:1-18.