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*

KRISTEN VAN GELDER

on Wednesday, April 28, 2021 at 1:30 p.m. (online)

Thesis Title: **Understanding the biosynthesis and function of polyisoprenoids in *Solanum lycopersicum***

Examination Committee:

Dr. Ray Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Tariq Akhtar, Dept. of Molecular and Cellular Biology
Dr. Gale Bozzo, Dept. of Plant Agriculture
Dr. Robert Mullen, Dept. of Molecular and Cellular Biology
Dr. Michael Phillips, Dept. of Biology, University of Toronto (External Examiner)

Advisory Committee:
Dr. Tariq Akhtar (Adv)
Dr. Jaideep Mathur
Dr. Gale Bozzo

Abstract: The occurrence of polyisoprenoids has been well documented over the past 60 years. Polyisoprenoids are lipophilic polymers of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) which are linked via a class of enzymes known as *cis*-prenyltransferases (CPTs). In *Solanum lycopersicum*, only dolichols of 75-85 carbons are known to exist, as they play a role in the post-translational modification of proteins. Polyprenols, the other major class of polyisoprenoids has yet to be identified in tomato. Using *S. lycopersicum,* which contains a seven member CPT gene family, two CPTs were studied for their role in the biosynthesis of polyprenols and dolichols. One such CPT (*Sl*CPT5) was identified for its role in catalyzing the formation of polyprenols of 45-55 carbons. Using a combination of *in vitro* and *in vivo* analyses, it was determined that *Sl*CPT5 resides in the plastid stroma where it catalyzes the biosynthesis of medium-chain polyprenols using geranylgeranyl diphosphate (GGPP) as a substrate. Furthermore, the use of RNAi-mediate knockdown of *Sl*CPT5 tomato plants demonstrated that an absence of polyprenols can lead to membrane and protein instability in the plastid and can hinder photosynthetic efficiency. When establishing a heterologous expression system for the biosynthesis of polyprenols, it was discovered that *Sl*CPT5 exhibits moonlighting activity as it is also involved in the biosynthesis of glycinoprenols. The glycinoprenols were of similar medium-chain lengths (40-50 carbons) but contained three saturated IPP units. Lastly, the above heterologous expression system was configured for the synthesis of dolichols by over-expressing the *Sl*CPT3 gene. It was shown that *Sl*CPT3 together with its partner protein, CPT binding protein (*Sl*CPTBP), catalyze the formation of polyprenols of 75-85 carbons in length. The addition of a polyprenol reductase (*Sl*PPRD) results in the biosynthesis of dolichols. In summary, this study demonstrates that two *S. lycopersicum* CPTs, namely *Sl*CPT5 and *Sl*CPT3, are required for the biosynthesis of medium- and long-chain polyprenols.

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Curriculum Vitae: Kristen completed their Bachelor of Science (Hons.) at the University of Guelph in the spring of 2016. They then began their MSc. in the lab of Dr. Tariq Akhtar in the fall of the same year and rolled up to a PhD in the winter of 2018.

Awards: National Science and Research Council of Canada – Postgraduate Scholarship (2019-2020)

Publications: **Van Gelder K**, Virta LKA, Easlick J, Prudhomme N, McAlister JA, Geddes-McAlister J, Akhtar TA. (2021) A central role for polyprenol reductase in plant dolichol biosynthesis. *Plant Sci. J.* 303:1100773.

Van Gelder K, Forrester T, Akhtar TA. (2020) Evidence from stable isotope labeling that catechol is an intermediate in salicylic acid catabolism in the flowers of *Silene latifolia* (white campion). *Planta* 252:1-11.

Van Gelder K, Virta LKA, Rea KA, Whitnell K, Osborn M, Vatta M, Khozin A, Svaikauskas F, Marangoni AG, Skorupinska-Tudek K, et al. (2018) Medium-chain polyprenols influence chloroplast membrane dynamics in *Solanum lycopersicum. Plant Cell Physiol.* 59:2350-2365.

Akhtar TA, Surowiecki P, Siekierska H, Kania M, **Van Gelder K**, Rea KA, Virta LKA, Vatta M, Gawarecka K, Wojcik J, et al. (2017) Polyprenols are synthesized by a plastidial *cis*-prenyltransferase and influences photosynthetic performance. *Plant Cell* 29:1709-1725.