ANNOUNCEMENT: Interested members of the University Community are invited to attend the Final Oral Examination for the Degree of Doctor of Philosophy of

Chevonne E. Carlow

of the Department of Molecular and Cellular Biology on Thursday, April 28, 2016 at 1:30 p.m. in SSC 2315

Thesis Title: Analysis of the Vitis CBF genes and their potential roles in the both the CBF and Stomatal Development Pathways

Examination Committee: Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Chair)
Dr. A. Nassuth, Dept. of Molecular and Cellular Biology
Dr. S. Rothstein, Dept. of Molecular and Cellular Biology
Dr. R. Lu, Dept. of Molecular and Cellular Biology
Dr. S. Gazzarrini, University of Toronto

ABSTRACT

Chevonne E. Carlow, B.Sc., M.Binf. Advisor: Dr. A. Nassuth

Vitis vinifera species are adapted for growth in moderate climates, so freezing temperature damage during Ontario winters results in significant revenue loss for producers. Highly conserved DREB1/CFB genes have been shown to be crucial in the acquisition of frost tolerance, while DREB2 genes were thought to function in drought tolerance. Seven CBF (C-repeat binding factor) genes and two DREB2 (DRE binding factor) genes were cloned from both Vitis vinifera and the more cold hardy Vitis riparia. Vitis CBFs showed differing expression patterns under ambient and cold conditions, with specific subsets expressed in leaf and bud tissues. Transcripts for the two DREB2 genes were not observed in conditions where CBFs had been detected. Transient transactivation assays showed that all Vitis CBFs but one and both DREB2 proteins could induce transcription via the core CRT/DRE promoter sequence element. Investigation revealed that the C-terminal hydrophobic domains present in CBF6 but absent from CBF5 contributed to activation. Predicted PEST motifs in both CBF4 and DREB2-3 affect activation by CBF4 only. Further investigation found that CBF and DREB2 proteins had a preference for the sequence surrounding the DRE core based on their classification; CBF proteins preferred an AT-rich sequence, while
DREB2 proteins preferred a GC-rich sequence. From the evidence presented in this thesis, we predict that *Vitis* CBFs and DREB2s have roles in different abiotic stress tolerance pathways, and that the individual members of each of these families have different functions within their respective regulons.

Evidence from transient transactivation and overexpression studies suggests that *Vitis* DREB2-5 and CBF8 may also have a role in the stomatal development pathway. Their overexpression induced a higher pavement cell density, similar to phenotypes previously found for *Vitis* SPEECHLESS and ICE4. We hypothesize that these four proteins are involved in the first step of the *Vitis* stomatal development pathway, the commitment of pavement cells to further development into stomata. How they do that remains unclear, but the work presented here provides a basis of understanding to help direct forthcoming investigations.

CURRICULUM VITAE:
Chevonne received her Bachelor of Science, double major in Biology and Medical Science from the University of Western Ontario in 2010. She received a Masters of Bioinformatics from the University of Guelph under the supervision of Dr. Lewis Lukens and Dr. Paul McNicholas in 2011. She began her graduate program in the lab of Dr. Annette Nassuth in September 2011.

AWARDS AND SCHOLARSHIPS:
Ontario Graduate Fellowship (2013-2014)
Registrar's Graduate Research and Travel Grant, University of Guelph (2013)
Ronald C. Moyer Graduate Scholarship, Grape Growers of Ontario (2012)

PUBLICATIONS:
Carlow, C. E. and A. Nassuth. 2016. Investigation of the DREB1/CFB transcription factor family in *Vitis vinifera* and *Vitis riparia*. (Manuscript in Preparation)