University of Victoria | Graduate Studies

Notice of the Final Oral Examination for the Degree of Doctor of Philosophy

of

CUONG LE

BSc (University of British Columbia, 2009)

"Hydroxycinnamoyl Transferases in *Populus* and their Roles in Vascular Development"

Department of Biochemistry and Microbiology

Monday, December 11, 2017
1:00 P.M.
Petch Building
Room 206

Supervisory Committee:
Dr. Christoph Borchers, Department of Biochemistry and Microbiology, University of Victoria (Co-Supervisor)
Dr. Juergen Ehlting, Department of Biochemistry and Microbiology, UVic (Co-Supervisor)
Dr. Caren Helbing, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Peter Constabel, Department of Biology, Uvic (Outside Member)

External Examiner:
Dr. Jim Mattsson, Department of Biology, Simon Fraser University

Chair of Oral Examination:
Dr. Timothy Iles, Department of Pacific and Asian Studies, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Hydroxycinnamoyl conjugates (HCC)s are an extremely diverse class of natural products that serve a wide variety of key functions in plant development, for example during wood formation, and in chemical ecology. They have diverse biological properties and act as antioxidants, antimicrobials, and antivirals. The biochemical basis of HCC diversity, however, has not yet been fully elucidated. Plants in the *Populus* genus are known to produce a particularly diverse range of HCCs and they constitute up to 5% of the leaf dry mass in some *Populus* species. HCCs can be formed by hydroxycinnamoyl transferases (HCTs) and many of the biochemically characterised HCTs belong to the BAHD superfamily of acyltransferases. My phylogenetic reconstruction of the BAHD superfamily has defined a subclass containing most of the already-characterised HCTs, including nine potential HCT candidates in *Populus*.

Caffeoyl-shikimate is a known precursor in the formation of lignin, the biopolymer that imparts mechanical stability to wood. Based on the expression profiling of two candidate genes HCTA-1 (Potri.001G042900)) and HCTA-2 (Potri.003G183900) were hypothesised to be responsible for caffeoyl-shikimate formation in secondary xylem (i.e., wood). As part of this project, RNAi whole-plant knockdowns were generated for the xylem-associated HCT-A1/A2. The HCT-A1/A2 RNAi knockdowns have stunted growth, reminiscent of other mutants with impaired lignin biosynthesis. Based on thioacidolysis GC-MS, I found that the mutants produced a lignin with enriched hydroxyphenyl (H) subunits, which were derived from precursors upstream of the HCT-catalysed reaction and normally do not occur in *Populus* lignin. Interestingly, in one of the RNAi lines, the lignin phenotype was uncoupled from the developmental dwarfing phenotype. This is of high interest from a bioethanol perspective, since wood rich in H-lignin is more easily fermented than wood that is rich in guaiacyl (G) and syringyl (S) lignin. Another candidate gene (Potri.018G109900, HCT-E2) was linked to the formation of caffeoyl-spermidine in male catkins (which function in pollen coat formation), and one candidate gene (Potri.018G104700, HCT-C2) was associated with the formation of bioactive, soluble HCCs in leaves and roots. Since RNAi-mediated down-regulation proved ineffective, CRISPR-based gene knock-out methodology was developed and utilised for the *Populus* hairy root system. Targeted knock-out mutants for the leaf-associated HCT-C2 were generated. HCC identity was determined by metabolite purification and subsequent MS/MS/MS from leaf extracts, and the metabolite concentrations were determined by LC-MS. A decrease in chlorogenic acid concentration was apparent in CRISPR hairy-root knockouts of HCT-C2 indicating that HCTC2 is involved in HCC biosynthesis and can directly produce chlorogenic acid.