University of Victoria | Graduate Studies

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

of

DAWEI MA

BSc (Fudan University, 2012)

"Functional Characterization of Flavonoid R2R3-MYB Activators and Repressors Transcriptional Regulators in Poplar"

Department of Biology

Wednesday, December 4, 2019
10:00 A.M.
Clearihue Building
Room B007

Supervisory Committee:
Dr. Peter Constabel, Department of Biology, University of Victoria (Supervisor)
Dr. Juergen Ehlting, Department of Biology, UVic (Member)
Dr. Armand Seguin, Department of Biology, Uvic (Member)
Dr. Chris Nelson, Department of Biochemistry and Microbiology, UVic (Outside Member)

External Examiner:
Dr. Kyung-Hwan Han, Department of Horticulture and Forestry, Michigan State University

Chair of Oral Examination:
Dr. Sara Ramshaw, Faculty of Law, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Flavonoids are important and ubiquitous secondary metabolites and are known to participate in various developmental and stress response processes in plants. Common flavonoids include anthocyanins, proanthocyanidins and flavonols. This thesis aims to determine, at the molecular level, how the biosynthesis of flavonoids, in particular the proanthocyanidins, is regulated in poplar. Poplars accumulate large amount of flavonoids and the major flavonoid biosynthetic genes in poplar have been identified. Flavonoid biosynthesis is known to be regulated by MYB transcription factors. Previous work had identified MYB134 as a key regulator of proanthocyanidin synthesis in poplar. Here I describe experiments on five additional genes encoding MYB activators (MYB115 and MYB117), MYB repressors (MYB165 and MYB194), and one bHLH cofactor (bHLH131) as possible flavonoid regulators in poplar. The objective of this work is to determine the *in planta* functions of these new flavonoid regulators using reverse genetic methods, phytochemical and transcriptome analysis, to identify their target genes and to determine how these transcriptional regulators interact using promoter transactivation and yeast twohybrid assays.

MYB115 was identified as a second proanthocyanidin regulator. Similar to the effects of MYB134, overexpression of MYB115 in poplar led to increased proanthocyanidin content and upregulated flavonoid biosynthesis genes, but reduced the accumulation of salicinoids.

Overexpression of repressor type MYBs, MYB165 or MYB194 led to reduced anthocyanin, salicinoid and hydroxycinnamic ester accumulation in leaves, while reducing proanthocyanidin content in roots. Transcriptome analysis demonstrated the downregulation of most flavonoid genes in these transgenics, as well as some shikimate pathway genes, confirming the broad repression function on the phenylpropanoid pathway.

By contrast, MYB117 encodes an anthocyanin activator, and was shown to be specific to this branch of the flavonoid pathway. Overexpression of MYB117 in transgenic poplar increased accumulation of anthocyanin in all tissues, resulting in red poplar plants.

One bHLH cofactor, bHLH131 was shown to interact with both MYB activators and repressors and required by MYB activators to activate flavonoid gene promoters. This indicate an important role of bHLH131 in the flavonoid biosynthesis.

Proanthocyanidin MYB activators, MYB134 and MYB115 could activate each other. This indicates a positive feedback loop of proanthocyanidin MYB activators. Interestingly, repressor MYB165 suppressed expression of other flavonoid MYB repressors including MYB194 and MYB182, which shows a negative feedback loop of MYB repressors. The expression of bHLH131 was also regulated by MYB activators and repressors. These results reveal the complex interaction between these regulators.

Unexpectedly, overexpression MYB134, MYB115 or MYB117 poplars upregulated flavonoid 3’5’-hydroxylase and cytochrome b5 genes, and lead to enhanced flavonoid Bring hydroxylation and an increased proportion of delphinidin, prodelphinidin and myricetin. MYB repressors downregulated flavonoid 3’5’-hydroxylase. Overexpression of flavonoid 3’5’-hydroxylase in poplar confirmed its function in enhancing B-ring hydroxylation. However, overexpression of cytochrome b5 in flavonoid 3’5’-hydroxylaseoverexpressing plants did not further increase flavonoid B-ring hydroxylation. Thus its role in flavonoid B-ring hydroxylation remained unclear. These results show that flavonoid MYBs could also alter flavonoid structure.

Together, these studies outline the complex regulatory network formed by flavonoid MYB activators and repressors, and bHLH cofactors controlling both flavonoid accumulation and structure.