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A systems genetics approach to identify candidate genes driving salicinoid diversity in *Populus tremula*

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Academic dissertation

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Abstract

Trees have evolved an impressive array of strategies to cope with the challenges of having a long and sessile life. Not only must they withstand a fluctuating climate, but they also face instantaneous pressures from herbivores and other attackers. To protect themselves, plants can produce defence compounds, many of which are highly specialised and taxon specific. Within the Salicaceae family, a key group of such defence compounds are the salicinoid phenolic glycosides (SPGs). Many structural variants of SPGs have been identified, in which acyl groups (e.g., cinnamoyl, benzoyl, and acetyl) are common. Some of these SPGs can have toxic and deterrent effects against attackers, and a few are known for their medicinal properties in humans. However, the biological function of most SPGs *in planta* remains unclear, and the causal enzymes for the majority of SPGs are yet to be identified.

The aim of this thesis was to uncover the genetic basis of SPG biosynthesis in European aspen (*Populus tremula* L.) and to determine the extent of ontogenic and organ-specific variation among individuals. To achieve this, SPG variation within a collection of natural aspen, the Swedish aspen (SwAsp) collection, was investigated using an integrative multi-omic approach. By analysing the metabolome and transcriptome of multiple leaf ages from aspen individuals with varying levels of cinnamoyl and acetylated SPGs, a set of candidate transferases and novel putative SPGs were identified. These analyses further suggested that young leaf tissue is a highly active site of SPG biosynthesis, compared with mature leaves.

To extend this analysis, we performed genome-wide association studies on transcriptomic and metabolomic data from leaf buds to identify genomic regions associated with variation in SPG abundance and gene expression. These data were integrated into a systems genetics network, visualising the intricate relationship between candidate genes and the diversity of SPGs. Among the candidates, an acyltransferase was highly associated with both acetyl- and cinnamoyl-SPGs. Heterologous expression assays in *Escherichia coli* (*E. coli*) confirmed its acetylation activity. In line with these findings, overexpression of the gene *in planta* led to increased levels of acetyl-SPGs, suggesting acetylation activity of the enzyme.

In summary, these results have enhanced our understanding of SPG biosynthesis and provide a foundation for future studies aimed at elucidating the *in planta* function of the remaining candidate genes.

Keywords: aspen | *Populus tremula* | systems genetics | GWAS | eQTL | metabolomics | specialised metabolites | salicinoid phenolic glycosides | chemotype | liquid chromatography-mass spectrometry | transcriptomics | RNA-Seq

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