University of Victoria | Graduate Studies

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

of

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"The Role of BAHD Acyltransferases in Poplar (*Populus* spp.) Secondary Metabolism and Synthesis of Salicinoid Phenolic Glycosides"

Department of Biology

Wednesday, April 15, 2015
1:00PM
David Turpin Building
Room A144

Supervisory Committee:
Dr. Peter Constabel, Department of Biology, University of Victoria (Supervisor)
Dr. Juergen Ehlting, Department of Biology, UVic (Member)
Dr. Rachael Scarth, Department of Biology, UVic (Member)
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Abstract

The salicinoids are phenolic glycosides (PGs) characteristic of the Salicaceae family and are known defenses against insect herbivory. Common examples are salicin, salicortin, tremuloidin, and tremulacin, which accumulate to high concentrations in the leaves and bark of willows and poplars. Although their biosynthetic pathway is not known, recent work has suggested that benzyl benzoate acts as a possible biosynthetic intermediate. We identified three candidate genes encoding BAHD-type acyltransferases that are predicted to produce benzylated secondary metabolites, named *PtACT47*, *PtACT49*, and *PtACT54*.

Expression of *PtACT47* and *PtACT49* generally correlated with PG content in a variety of plants tissues and organs of wild type hybrid poplar plants. This relationship was also present in transgenic hybrid poplar overexpressing the condensed tannin regulator protein MYB134. In these plants, a suppression of *PtACT47* and *PtACT49* expression was correlated with lower PG content. In contrast, *PtACT54* exhibited very low expression in wild type plants across all tissues tested, and this level expression was not affected in MYB134 plants.

In order to better understand their biochemical activities, cDNA cloning, heterologous expression, and *in vitro* functional characterization was performed on these three BAHD acyltransferases. Recombinant PtACT47 exhibited a low substrate selectivity and could utilize acetyl-CoA, benzoyl-CoA, and cinnamoyl-CoA as acyl donors with a variety of alcohols as acyl acceptors. This enzyme showed the greatest *K*m/*K*cat ratio (45.8 nM-1 sec-1) and lowest *K*m values (45.1 *μ*M) with benzoyl-CoA and salicyl alcohol, and was named benzoyl-CoA:salicyl alcohol *O*-benzoyltransferase (PtSABT). Recombinant PtACT49 utilized a narrower range of substrates, specifically benzoyl-CoA and acetyl-CoA and a limited number of alcohols. Its highest *K*m/*K*cat (31.8 nM-1 sec-1) and lowest *K*m (55.3 *μ*M) was observed for benzoyl-CoA and benzyl alcohol, and it was named benzoyl-CoA:benzyl alcohol *O*-benzoyltransferase (PtBEBT). Both enzymes were also capable of synthesizing plant volatile alcohol esters at trace levels, for example hexenyl benzoate. Recombinant PtACT54 shares low sequence identity with PtSABT (52.3%) and PtBEBT (52.5%) and exhibited only moderate BEBT-like properties, being able to synthesize benzyl benzoate from benzoyl-CoA and benzyl alcohol at markedly lower level than PtBEBT. PtSABT and PtBEBT appear to be paralogs based on their high sequence identity (90.6%) and closely related yet distinct biochemical functions. They likely arose from gene duplication and subsequent functional diversification possibly by neofunctionalization.

Wounding experiments on wild type hybrid poplar showed that abiotic damage stimulated the synthesis of specific PGs, notably salicin and salicortin within 24-48hrs. This was accompanied by a proportional increase in the expression of *PtSABT* and *PtBEBT*. Furthermore, experiments using transgenic RNAi hybrid poplar lines with knock-down suppression of *PtBEBT*, and *PtSABT*, and both genes simultaneously, provided the first direct evidence that BAHD acyltransferases are important in PG production. *PtSABT* suppression, both individually and in the double knock-down suppression, significantly lowered salicortin content, particularly in mature leaves. However, a reduced level of *PtBEBT* expression did not have a significant effect on the PGs measured. This could indicate that BEBT-like activity, and in particular the production of benzyl benzoate, may be a redundant or shared function among closely related BAHDs. The manufacture of transgenic plants with suppression of multiple *BEBT*-like genes may be necessary to further delineate their functions. Future work may also include coexpression analysis studies of *PtSABT* and *PtBEBT* that could identify other genes potentially linked to the PG biosynthetic pathway(s).