

102. Inducible Extreme Expression of Cellulases in Poplar

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Project Goals: The overall goals of the project is to verify in poplar the In Plant Activation (INPACT) technology¹, which enables inducible expression of genes and accumulation of proteins at very high levels *in planta* and to evaluate the ability of cellulases produced through this technology to hydrolyze cellulose to simple sugars for fermentation.

Cost of enzymes for biofuel production is one of the major limitations for the widespread use of biofuels from lignocellulosic biomass. Production of enzymes *in planta* would decrease the amount of additional enzymes necessary for hydrolysis of cellulose. In Plant Activation technology allows for very high inducible expression of recombinant proteins *in planta*.

INPACT uses the rolling circle replication of Gemini virus to produce high levels of gene amplification and protein production. In this project, we will verify the adaptability of this technology in poplar to accumulate proteins at very high levels. Using this technology, we will express cellulases in poplar with constitutive and tissue specific promoters. Cellulases from three major groups of enzymes, endoglucanases, exoglucanases and β -glucosidases, involved in the hydrolysis of cellulose will be expressed with constitutive and tissue specific promoters.

Cellulases from thermophilic organisms will be codon optimized and cloned into INPACT vectors for transformation and expression at very high levels in poplar. The expression of INPACT system will be controlled by the alcohol-inducible transgene expression system. The yield and hydrolytic activity of the cellulases produced will be tested.

References

Dugdale, Benjamin, et al. "In Plant Activation: An inducible, hyperexpression platform for recombinant protein production in plants." *The Plant Cell Online* 25.7 (2013): 2429-2443.

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