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) technology, 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 cellulolytic enzymes is a significant constraint in biofuel production from lignocellulosic biomass. *In planta* production of these enzymes could potentially reduce the cost associated with bioethanol production. This project aims to produce high levels of cellulases in poplar upon induction with an exogenous chemical. Production of enzymes *in planta* would decrease the amount of additional enzymes necessary for hydrolysis of cellulose. In Plant Activation (INPACT) 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 initially, we will verify the adaptability of this technology in poplar for its ability to accumulate recombinant proteins at very high levels. We will then use INPACT to express cellulases from three major groups of enzymes, endoglucanases, exoglucanases and β-glucosidases in poplar with constitutive and tissue specific promoters. Cellulases from thermophilic organisms have been plant codon optimized and synthesized. These cellulases have been assessed for correct splicing in tobacco and protein production is currently being assessed in yeast. The constructs harboring the alcohol inducible replication initiation protein (Rep) which allows for the induction of the INPACT system has been successfully transformed into poplar and a mother line selected based on Rep/RepA gene expression and plant growth before and after alcohol treatment. The transgenic poplar plants with the alcohol inducible Rep and the GUS gene in the split orientation are being multiplied to produce clonal lines and will then be evaluated for expression in leaf and developing xylem using the GUS reporter system. The transgenic mother plants with thermostable cellulases in the split orientation are being regenerated, as are the positive and negative control lines.

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