

93. Inducible Extreme Expression of Enzymes 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.

One of the major limitations for the cost-effective production of biofuels is the cost of cellulolytic enzymes. One potential solution is the in planta production of these enzymes within the biofuels crop itself. Utilizing In Plant Activation technology (INPACT), which uses the rolling circle replication of the Gemini virus to produce high levels of gene amplification and protein production under an inducible promoter, this project aims to produce high levels of cellulases in poplar upon induction. Initially, we will verify this technology in poplar for its ability to accumulate recombinant proteins at very high levels. We will then use the technology to express cellulases from three major groups, 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 are being assessed for correct splicing in yeast. The construct harboring the alcohol inducible promoter driving the production of the replication initiation protein (Rep) which causes induction of the INPACT system has been successfully transformed into poplar and mother plants selected based on Rep/RepA gene expression and plant growth before and after alcohol treatment. Mother plants with the highest Rep gene expression after induction and with no expression prior to induction, are currently being transformed with the split gene cassette to assess the INPACT system in poplar. The transgenic poplar plants with the complete INPACT system are being regenerated and will be evaluated for expression in leaf and developing xylem using the GUS reporter system.

References

1. 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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