Extreme Inducible Expression of Cellulases in Poplar

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Project Goals: The overall goals of the project are to verify in poplar In Plant Activation (INPACT) technology[1], 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.

Cellulolytic enzymes are used to hydrolyze carbohydrates within lignocellulosic biomass into fermentable sugars, and the cost of these enzymes significantly increases the cost of bioethanol. An alternative is the production of these enzymes *in planta* in the lignocellulosic biomass itself. This would allow a single feedstock to play a dual role as both biomass substrate and enzyme provider. The aim of this project is to produce high levels of these cellulolytic enzymes in poplar using In Plant Activation (INPACT) technology. INPACT technology allows for very high inducible expression of recombinant proteins *in planta*. INPACT uses the rolling circle replication seen in Gemini viruses to produce high levels of gene amplification and protein production. 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.

To date, cellulases from thermophilic organisms have been codon optimized and cloned into INPACT vectors. Constructs harboring the alcohol inducible replication initiation protein (Rep) which allows for the induction of the INPACT system have been successfully transformed into poplar and a mother line selected based on Rep/RepA gene expression and plant growth before and after alcohol treatment. GUS constructs in the split orientation were then super-transformed into the mother line to generate INPACT-GUS double transgenic lines. Independent transgenic poplar lines harboring both the alcohol inducible Rep construct and the split orientation GUS gene were then multiplied to produce clonal lines. Currently these INPACT-GUS lines are being evaluated in the greenhouse for expression in leaf and developing xylem using the GUS reporter system. Transgenic plants with thermostable cellulases in the split orientation have been super-transformed in the Alc-Rep mother line to generate INPACT-Cellulase double transgenic lines, along with corresponding positive and negative control lines. These lines are presently being multiplied for greenhouse evaluation.

References

1. Dugdale, Benjamin, et al. "In plant activation: an inducible, hyperexpression platform for recombinant protein production in plants." The Plant Cell 25.7 (2013): 2429-2443.

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