Post-harvest Induction of Hyperthermophilic Cellulases Reduces Recalcitrance 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 glucose for fermentation. In addition, we aim to identify transgenic lines that have improved processability due to the accumulation of cellulases, either through traditional expression or through the use of the INPACT system.

Plant cell walls provide a vast, untapped source of sugars in the form of cellulose available for fermentation into ethanol and other biofuels. There remain several challenges in the attempt to developing economically viable cellulosic ethanol based on biochemical conversion, including the cost of cellulolytic enzymes and thermochemical pretreatments to reduce recalcitrance of the cell wall. One potential approach to reduce enzyme cost is the production of the enzymes within the feedstock itself. This would require a system that prevents the degradation of the cell wall until the desired time.

Here we verify in poplar a transgenic technology (In Plant Activation Technology – INPACT) that allows for the controlled production and accumulation of enzymes within the plant. This system is also being used to drive the expression of cellulases in poplar, with the transgenic lines currently being grown in the greenhouse.

When paired with a cellulose synthesis promoter, driving gene expression in secondary cell wall of vascular tissue, INPACT drives inducible expression of the GUS reporter gene. However, the level of expression does not reach 'extreme' levels at this time. It is likely that the improvement of the system, as carried out previously[2] will be necessary to drive high-level accumulation of enzymes.

While carrying out analysis of these INPACT lines, we have also assessed traditional overexpression lines with the Cauliflower Mosaic Virus 35S promoter driving the expression of cellulase genes. In particular, we have assessed a line expressing a hyperthermophilic *Thermotoga neapolitana* endoglucanase (TnCelB). Poplar-derived TnCelB retains high activity to substrates at 100°C, and in all transgenic lines, biomass was significantly increased. While there were some alterations in the plant cell wall composition, the line with the highest TnCelB activity showed enhanced glucan saccharification efficiency over WT with and without a post-harvest heat treatment. The same transgenic line showed gains in saccharification over WT without a traditional pre-treatment, solely from a heat treatment of 100°C immediately following harvest. This set of plants provided a feedstock substrate that yields increased glucose without a chemical pretreatment, solely through the activation of a hyperthermophilic cellulase.

Overall, this project has yielded evidence that the INPACT system is viable in poplar, though it will require additional fine-tuning. In addition, the control lines from the project, which are traditional overexpression lines, have yielded significant results in terms of improved glucose release without a chemical pretreatment.

References

1. Dugdale, B. *et al*. (2013) In plant activation: an inducible, hyperexpression platform for recombinant protein production in plants. The Plant Cell 25: 2429-2443.
2. Kinkema, M. *et al*. (2014) An improved chemically inducible gene switch that functions in the monocotyledonous plant sugar cane. Plant Molecular Biology 84:443-454.

Funding statement: This material is based upon work supported by the Department of Energy under Award Number DE-SC0010411.