

Modified Cell Wall Composition through Expression of an Expansin-Like Protein in Poplar

Yao Xiao,¹ Maria Dombrov,¹ Linnea Ritchie,¹ Charleson Poovaiah, and **Heather D. Coleman**^{1*} (hcoleman@syr.edu)

¹Syracuse University, Syracuse, NY

<http://asfaculty.syr.edu/pages/bio/Coleman-Heather.html>

Project goal: One of the challenges associated with the expression of cellulolytic enzymes *in planta* is the alteration of cell wall chemistry, and the resultant negative effects on plant growth and biomass production. Here we express a fungus expansin-like protein in poplar, with the intent of altering cell wall structure without compromising cell wall functions and plant growth.

Degradation of cellulose to glucose for ethanol production from lignocellulosic biomass requires multiple cellulase and accessory enzymes. One of the challenges with expressing these enzymes *in planta* is a potential resultant negative phenotype. By selecting enzymes that are known to disrupt structure without eliciting a ‘breakdown’ of cellulose, it is anticipated that phenotypes with improved hydrolysis but without detrimental phenotypes may be achieved. One such potential enzyme is the expansin-like protein Swollenin 1 (Swo) from *Trichoderma reesei*. Swo has been shown to disrupt cell wall structure without a corresponding release in carbohydrate monomers [1], and to work synergistically with hydrolytic cellulases [2]. Our work explores the effect of *in planta* expression of Swo on plant cell wall chemistry and structure, while investigating the potential of using this transgenic biomass in the production of cellulosic ethanol. The gene encoding Swo has been expressed in poplar under either the constitutive Cauliflower Mosaic Virus 35S promoter or the putative vascular tissue specific Subterranean Clover Stunt Virus S7 promoter. Four CaMV lines and three SCSV lines were grown in the greenhouse for four months along with corresponding wildtype controls. While there was little variation in growth phenotype, preliminary cell wall analysis by thermogravimetric analysis suggested large changes in cellulose and hemicellulose levels. Further analysis of cell wall chemistry is ongoing, as is assessment of cell wall crystallinity and amenability of biomass to enzymatic hydrolysis.

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1. Saloheimo, M., et al., *Swollenin, a Trichoderma reesei protein with sequence similarity to the plant expansins, exhibits disruption activity on cellulosic materials*. European Journal of Biochemistry, 2002. **269**(17): p. 4202-4211.
2. Gourlay, K., et al., *Swollenin aids in the amorphogenesis step during the enzymatic hydrolysis of pretreated biomass*. Bioresource technology, 2013. **142**: p. 498-503.