Southeastern Sun Grant Center Quarterly Progress Report

Project Title: A novel approach to facilitate accessibility of cellulose and hemicellulose: characterization of hybrid poplar transformed with a tyrosine-rich peptide gene

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1. Planned Activities:
   A. Thioacidolysis assay to determine syringyl to guaiacyl ratios in the stem tissues of transformed and wild-type poplar plants;
   B. Immunolocalization assay to demonstrate the presence of tyrosine-rich peptides in the cell walls of transformed tissues;
   C. Disease resistance tests to determine whether the introduction of a tyrosine-rich peptide gene would compromise tree health;
   D. Wood physical property analysis to compare tensile strength of the wood chips from transformed and wild-type poplar plants;
   E. Comparison of lignin digestibility with and without protease treatment between transformed and wild-type poplar tissues.

2. Actual Accomplishments:
   A. We have acclimated micro-propagated (tissue cultured) transgenic and wild-type poplar plants in potting mix and grew them in greenhouse. These materials are for pathogen tests, in vitro digestibility, immunolocalization, and wood tensile strength studies.
   B. We have initiated antibody production for the tyrosine-rich peptide thru Promab Biotechnology, Inc. Overexpression of the peptide gene was done in E.coli and two injections of the peptide have been done to five mice;
   C. We requested USDA-APHIS ePermit to get poplar pathogen Septoria musiva from Wisconsin. The pathogen is now being cultured for conidia collection for pathogen tests;
   D. Wood tensile strength was analyzed with a Dynamic Mechanical Analyzer DMA 2980 on wildtype and five transgenic poplar plants. We included two different stem samples for each transgenic line, each from the same sapling. Our preliminary data indicated reduced tensile strength in transgenic lines (Fig.1), except in transgenic line T22 (α=0.05, as tested by ANOVA with the Tukey pairwise comparisons test);

   ![Fig.1. Dynamic mechanical analysis data showing storage modulus results from wildtype and transgenic hybrid poplar. Bars represent means ± standard deviation (SD).](image-url)
E. We tested the digestibility of the stem tissue using a sequential treatment of protease K followed by cellulase/hemicellulase. The concentration of reducing sugar in each sample was detected by tetrazolium blue method and represented as milligrams of reducing sugar per milliliter per gram of stem tissue. Seven transgenic lines were included, and there were at least two experimental repeats for each sampling plant. Of the lines we surveyed, two (lines T1 and T7) showed significant differences in the amount of sugar released from stem digestions pre-treated with protease K, relative to those without protease treatment (Fig. 2) (p=0.0017, 0.0380, respectively for T1 and T7). In one of these cases (line T1), the digestibility of the non-protease treated stem tissue was similar to the wildtypes. However, in most of the transgenic lines, significantly more polysaccharides were released than with wildtype, independent of protease K treatment.

![Graph showing reducing sugar concentrations in stem tissue extracts of hybrid poplar “Ogy” wildtypes and transgenic lines. Each line represents a portion of ground tissue incubated with sequential incubations of protease K followed by cellulase and hemicellulase (shaded bars), while another portion was incubated only with cellulase and hemicellulase (open bars). Bars are means + SD of 2-3 replicates of individual saplings.]

3. **Explanation of Variance:**

A. Due to lack of stem tissues, tensile strength analysis and digestibility assay were only conducted on a few transgenic lines. We are planning to test more transgenic lines when materials become available. Only two transgenic lines showed more susceptibility to protease digestion as predicted. Most of the transgenic lines released more sugar even without protease digestion, just by digestion with cellulase and hemicellulases. This is not consistent with our predictions. Decreased tensile strength was also found in most of the surveyed transgenic lines. Without evidence of protein (the tyrosine-rich peptide) to lignin linkages through tyrosine, or protein linkage to anything else, it is not clear what caused the phenotypes observed in the digestibility assay and tensile strength analysis. We will test if the tyrosine-rich peptide was in the wall and associated with lignin by using NMR.

B. Antibody production for the tyrosine-rich peptide is approximately three months behind schedule because of the difficulty to express this peptide in E.coli.

C. We decided not to conduct thioacidolysis assay to determine syringyl to guaiacyl ratios in the stem tissues of transformed and wild-type poplar plants, since syringyl to guaiacyl ratio is not likely to change in the transgenic lines and not a primary concern in this project.

4. **Plans for Next Quarter:**
A. Test the digestibility and tensile strength in the remaining transgenic lines.
B. Conduct disease susceptibility assay with *Spetoria musiva*.

**Patents:** None

**Publications / Presentations:** one manuscript (“A novel approach toward lignin modification to facilitate cellulosic ethanol production: introducing a tyrosine-rich cell wall peptide gene in poplar”, by Haiying Liang, Christopher J. Frost, Xiaoping Wei, Nicole R. Brown, John E. Carlson, Ming Tien) was submitted to journal of CLEAN - Soil, Air, Water.