

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

Recipient Organization: Clemson University, South Carolina

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Project Location: Clemson University, South Carolina

Reporting Period: July 1, 2008 – Dec. 31, 2008

Date of Report: March 5, 2009

Written by: Haiying Liang

1. Planned Activities:

- A. Finish the digestibility and tensile strength assays in the remaining transgenic lines.
- B. Finish the Western blot analysis and immunolocalization of the transgene product;
- C. Finish disease susceptibility assay with *S. musiva*.
- D. Complete a portion of the NMR experiments
- E. Conduct herbivore challenge tests.

2. Actual Accomplishments:

A. Assessment of overall lignin, cellulose, and hemicellulose content

Seven transgenic lines carrying the tyrosine-rich peptide gene as well as wild-type plants were characterized in terms of lignin, cellulose, and hemicelluloses contents by sequential digestion using an Ankom fiber analyzer. It appeared that expression of the transgene did not affect the lignin, cellulose, and hemicelluloses contents dramatically (Figure 1), which is consistent with our hypothesis. This work was done by an independent laboratory at Penn State.

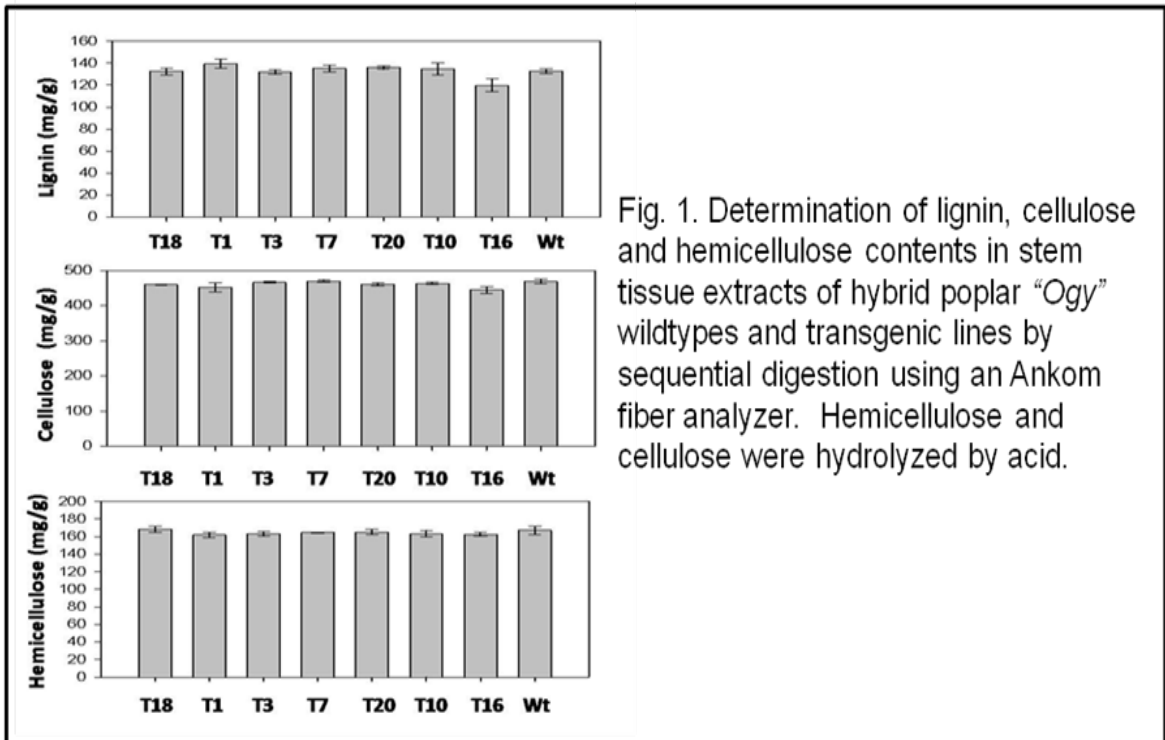


Fig. 1. Determination of lignin, cellulose and hemicellulose contents in stem tissue extracts of hybrid poplar "Ogy" wildtypes and transgenic lines by sequential digestion using an Ankom fiber analyzer. Hemicellulose and cellulose were hydrolyzed by acid.

B. Protein immunolocalization

Protein immunolocalization was observed by transmission electron (TEM) microscopy, as shown in Figure 2. Small dark dots indicate gold particles which recognize TYR protein. No much difference was observed between wild-type tissues and transgenic counterparts. Low antibody specificity and/or low transgene expression at translational level could be causes. Another possibility could be that phenolic hydroxyls of tyrosine in the protein were linked into lignin complex which prevented the recognition of antibody. The second generation of transgenic poplar with His-tag will be used to conduct this assay again.

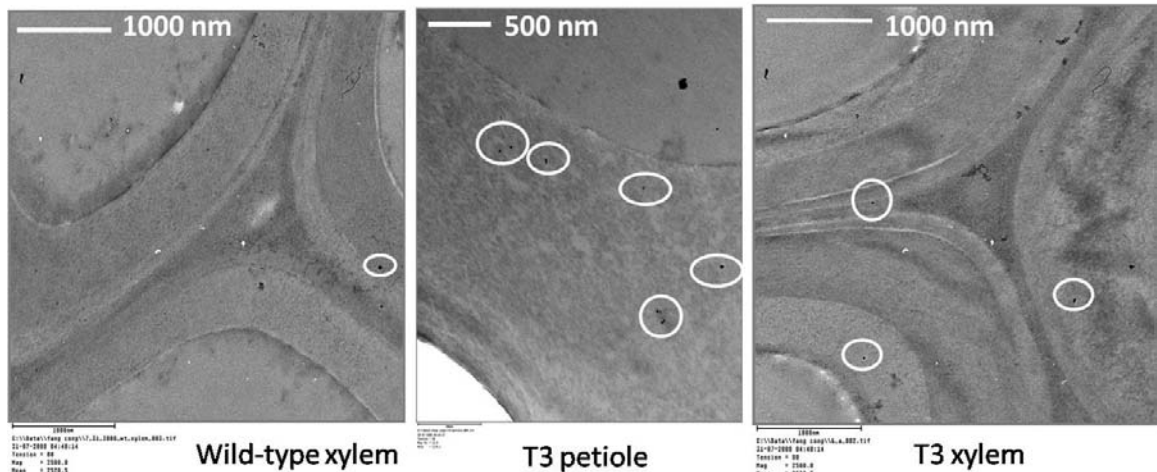


Fig.2. Protein immunolocalization by transmission electron

C. Pathogen susceptibility assay

Leaf discs 15 mm in diameter were inoculated with *S. musiva* conidia and incubated at 22 ± 2 °C under a 16 h photoperiod of cool-white fluorescent light (photon flux of $35 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 4 weeks. Necrotic areas of the leaf discs were then measured by manually outlining the necrosis using the NIH Image 1.61 program. Leaf discs inoculated with 20 μl of sterile water were used as negative controls. The results of ANOVA showed that there were no significant difference between the wild types and transgenic plants at the probability level of 0.05 (p-value of ANOVA=0.8592) (Figure 3).

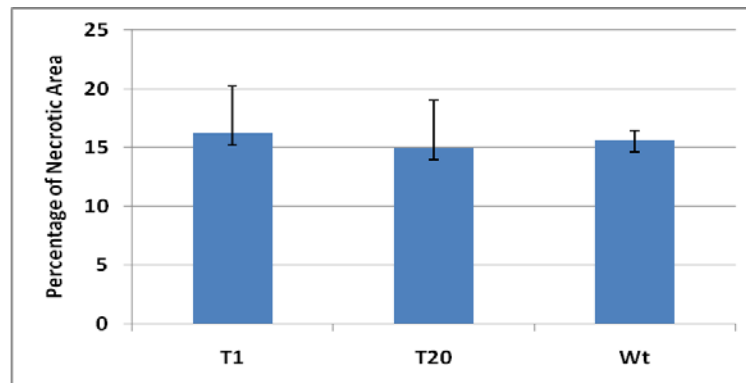


Fig. 3. Percentage of necrotic area in leaf discs inoculated with *S. musiva*. The error bars represent the standard deviations.

3. Explanation of Variance:

- A. Due to lack of plant materials, herbivore tests were not initiated. We will conduct this experiment when enough plant materials become available.
- B. NMR experiments were not initiated because there was not enough funding for this work.

4. Plans for Next Quarter:

The grant ended on Dec. 31, 2008. However, we are continuing to finish the pathogen and herbivore tests on the current transgenic poplars and compare the growth rate between transgenic and wild-type plants. We are also generating transgenic poplar carrying our next generation version of TYR-rich peptide constructs, which contain a histidine tag. In addition, we are interested in dissecting the molecular mechanisms underlying the more flexible and more digestible cell wall that we observed in our transgenic poplar by microarray analysis when funding is available.

Patents: None

Publications / Presentations:

- One manuscript published: Haiying Liang, Christopher J. Frost, Xiaoping Wei, Nicole R. Brown, John E. Carlson, Ming Tien (2008) A novel approach toward lignin modification to facilitate cellulosic ethanol production: introducing a tyrosine-rich cell wall peptide gene in poplar. *CLEAN - Soil, Air, Water*, 36 (8): 662-668.

Financial:

1. *Cummulative Expenditures to Date:* **\$13,3095**
2. *Remaining Balance:* **None**