Comparative Genomics Guided Genetic Modification of Switchgrass Cell Wall for Improved Lignin Characteristics and Increased Cellulose Production

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1. **Planned Activities**: Grow rice to generate enough plant material for the analysis of lignin down-regulation in rice. Grow 20 Arabidopsis lignin biosynthesis mutants and analyze the biomass content and composition. Finish the switchgrass constructs and begin transformation. We will begin tissue preparation for transformation. Complete the manuscript for comparative and functional genomics analysis of lignin biosynthesis gene family.

2. **Actual Accomplishments:** Obtain the *C3H* down-regulation rice transgenic lines, and continue to grow to generate enough plant material for the analysis of chemical composition. Planted 20 Arabidopsis lignin biosynthesis mutants and keep growing those plant materials to analyze the biomass content and lignin composition. By searching the ESTs' library released for PAL (phenylalanine ammonia lyase) gene with PALs in rice and poplar, we got 21 EST tags for PAL in Siwtchgrass, in which could be assembled into 7 unique genes with software Vector NTI. Two RNAi fragments referred to different sequences were chosen and subcloned into pCR@8/GW TA vector. The construct of RNAi vector for PAL down-regulation was carrying out.

   Get the preliminary analytical data of chemical composition of the various parts (leaves and internodes) of switchgrass grown in the field and in the greenhouse. Based on the samples analytical results, C6 sugars in leaves and internodes from greenhouse are a somewhat higher than those from field, which seems that sugar content biosynthesized and accumulated in greenhouse is larger than those in the field. The spectroscopic analytical results show the difference of the lignin contents from greenhouse and field.

   See details following.

3. **Explanation of Variance**: Due to the redesign of the RNAi construct for lignin down-regulation in switchgrass, the progress of transformation of switchgrass could be delayed.

4. **Plans for Next Quarter**:
   a. Finish the construct of RNAi vector for PALs in Switchgrass.
   b. Begin to transform the Switchgrass and screen for the positive transgenic lines.

c. Obtain the corresponding transgenic lines of Switchgrass and rice based on the different RNAi down-regulation strategies.
d. Finish the measurement of the lignin, cellulose composition and biomass data in those transgenic lines.
e. Screen for any relationship between those data and RNAi down-regulation designs and optimize the lignin pathway redirection.
f. Complete corresponding papers or patents for those RNAi transgenic lines generation.

Patents: None during the period.

Publications / Presentations: None during the period.