This project aims to identify the regulatory genes of rice cell wall synthesis pathways using a cell wall removal and regeneration system.

We completed the gene expression profiling studies following the time course from cell wall removal to cell wall regeneration in rice suspension cells. We also completed, total proteome, nuclear subproteome and histone modification studies following the course from cell wall removal and cell wall regeneration process. A large number of differentially expressed regulatory genes and proteins were identified. Meanwhile, we generated RNAi and over-expression transgenic rice for 45 genes with at least 10 independent transgenic lines for each gene. In addition, we ordered T-DNA and transposon insertion mutants for 60 genes from Korea, Japan, and France and characterized the mutants. Overall, we have mutants and transgenic lines for over 90 genes, exceeded our proposed goal of generating mutants for 50 genes.

Interesting Discoveries

a) Cell wall re-synthesis in protoplast may involve a novel mechanism. The synthesis of the primary cell wall is initiated in late cytokinesis with further modification during cell expansion. Phragmoplast plays an essential role in cell wall synthesis. It services as a scaffold for building the cell plate and formation of a new cell wall. Only one phragmoplast and one new cell wall is produced for each dividing cell. When the cell wall was removed enzymatically, we found that cell wall re-synthesis started from multiple locations simultaneously, suggesting that a novel mechanism is involved in cell wall re-synthesis. This observation raised many interesting questions, such as how the starting sites of cell wall synthesis are determined, whether phragmoplast and cell plate like structures are involved in cell wall re-synthesis, and more importantly whether the same set of enzymes and apparatus are used in cell wall re-synthesis as during cytokinesis. Given that many known cell wall synthesis pathway genes are induced by removal of cell wall, some cell wall synthesis apparatus must be shared in both cases. The cell wall re-synthesis mechanism may have broad application because our preliminary assay indicates that the cell wall characteristics are highly different from those produced during cytokinesis. A thorough understanding on the regulation of cell wall re-synthesis may lead to improvement of cell wall characteristics.

b) Removal of cell wall results in chromatin decondensation

Another interesting observation was that removal of cell wall was associated with substantial chromatin change. Our DNA DAPI stain, chromatin MNase digestion, histone modification proteomics, protein differential expression analysis, and DNA oligo array studies all supported that substantial chromatin change was associated with removal of cell wall treatment. It is still under investigation if the chromatin change is associated with activation of cell wall synthesis genes, in which chromatin remodeling is required. Another possibility is that the cell wall is required for stabilizing the chromatin structure in plant cells. Given that spindle fiber is directly connected with both chromatin structure and cell wall synthesis, it is possible that there is an intrinsic connection between cell wall and chromatin.

6) Variances: We have completed or overly completed most of the four objectives proposed in our original proposal. The discoveries that cell wall re-synthesis in protoplasts is likely involve a novel regulatory mechanism of cell wall synthesis and that removal of cell wall leads to chromatin state change has lead to the shift of our research focuses. These two discoveries were truly exciting. However, they also resulted in delay of our publications. Although all reviewers are excited about

our observations, they request more evidence to support our conclusions. Consequently the publications were delayed. Nevertheless, more exciting publications are expected.

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Invited Presentations

Zhaohua Peng (2008) Identification of cell wall synthesis regulatory genes controlling biomass characteristics and yield in rice (Oryza sativa). International Plant and Genome Conference XVI, W358. Jan 12-16, San Diego, CA.

Zhaohua Peng (2009) Identification of Cell Wall Synthesis Regulatory Genes Controlling Biomass Characteristics and Yield in Rice (*Oryza sativa*) DOE-BER Genomics:GTL and USDA-DOE Plant Feedstock Genomics awardee meeting. Feb. 8, Bethesda, Maryland.

Zhaohua Peng (2011) Role of Histone Modifications in the Regulation of Cell Wall Synthesis in Rice (*Oryza sativa*). 2011 Genomic Science Annual Contractor-Grantee Meeting/USDA-DOE Plant Feedstock Genomics for Bioenergy Program Meeting. April 10-13, Arlington, VA.

Zhaohua Peng (2011) Role of Vacuolar Protein Bim62 in the Regulation of Cell Wall Characteristics in Arabidopsis. May 30, Jiangsu University, Zhenjiang, China.

Zhaohua Peng (2011) Role of Histone Modifications in the Regulation of Cell Wall Characteristics in Rice (*Oryza sativa*). May 31, Jiangsu Agricultural Academy of Science. NanJing, China.

Zhaohua Peng (2011) Role of Histone Modifications in the Regulation of Cell Wall Characteristics in Rice (*Oryza sativa*). June 23, Jiangsxi Agricultural University, Nanchong, China.

Zhaohua Peng (2011) Role of Vacuolar Protein Bim62 in the Regulation of Cell Wall Characteristics in Arabidopsis. June 24, Jiangxi Agricultural Academy of Science, Nanchong, China.

Proceedings

Peng, Z., Wang, G., Mujahid, H., Ronald, P., Tan, F. (2009). Analysis of the putative pathways involved in cell wall synthesis in rice (Oryza sativa) protoplasts. P213. The Future of Biofuels (D4) (The Keystone Symposia Biofuel Meeting); Snowbird, Utah; April 4 - April 8.

Zhaohua Peng, Pamela Ronald, Guo-liang Wang (2009) Identification of Cell Wall Synthesis Regulatory Genes Controlling Biomass Characteristics and Yield in Rice (*Oryza sativa*) DOE-BER Genomics:GTL and USDA-DOE Plant Feedstock Genomics awardee meeting. Feb. 8, Bethesda, Maryland.

Hana Mujahid, Feng Tan, Ki-Hong Jung*, Jian Zhang, Pamela Ronald*, Zhaohua Peng (2009) Identification of Cell Wall Synthesis Regulatory Genes Controlling Biomass Characteristics and Yield in Rice (Oryza sativa). Mississippi State University Biofuels Conference. August 6-7. Jackson, Mississippi.

Feng Tan, Kangling Zhang, Guo-Liang Wang, Pamela Ronald, and Zhaohua Peng (2010) Removal of the plant cell wall results in chromatin reorganization and differential acetylation of H3K18 and H3K23. Jan 9-13, International Plant & Animal Genome XVIII Program., San Diego, CA.

Zhaohua Peng (2011) Role of Histone Modifications in the Regulation of Cell Wall Synthesis in Rice (*Oryza sativa*). 2011 Genomic Science Annual Contractor-Grantee Meeting/USDA-DOE Plant Feedstock Genomics for Bioenergy Program Awardee Meeting. April 10-13. Arlington, VA.

Hana Mujahid, Feng Tan, and Zhaohua Peng, (2012) Protein Differential Expression and Modification in Response to Cell Wall Removal and Regeneration in Rice (Oryza sativa). Rice Technical Working Group 34rd Meeting, February 27-March 1, Hot Springs, Arkansas.