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Forward Genetic Screen to Identify Rice Mutants with Changes in Cell Wall Composition and Saccharification Efficiency

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Understanding plant cell wall biosynthesis is crucial for the development of the next generation of biofuels derived from lignocellulosic material. Current limitations in the harvest of fermentable sugars from cellulose derive from the inherent recalcitrance of plant cell walls. Basic knowledge of how the structure and composition of the cell wall can be modified to obtain biomass suitable for efficient and economically viable biofuel production is needed. We are using a forward genetics approach to identify genes responsible for cell wall characteristics affecting cell wall composition and deconstruction. By means of fast neutron mutagenesis, we have generated a rice mutant population consisting of 6,500 M0 lines and harvested more than 100,000 M1 seed from approximately 4,000 M0 plants. Leaves and stems from these lines have been collected and we are in the process of screening them for alterations in saccharification efficiency and cell wall composition. To screen for changes in fermentable sugar release from biomass, we have optimized a protocol using either hot water or dilute acid pre-treatment followed by enzymatic saccharification for adaptation into a 96 well format. In addition, we are standardizing a high throughput microfluidics platform for analyzing alterations in the C5/C6 monosaccharide ratios of total sugar extracts from leaves to identify mutants with changes in cell wall composition. We have also validated a method for pre-screening intact dried leaf tissue using Near Infrared spectroscopy to identify outliers in the mutant population that will be then analyzed using the microfluidics system. Once cell wall mutants are confirmed, we will extract DNA from wild type and highly prioritized mutant candidates and then carry out whole genome comparative hybridization on rice tiling arrays. This approach will allow us to identify genes in deleted region responsible for the mutant phenotypes. Mutants will be complemented with candidate genes using transgenic analysis and assayed for restoration of the cell wall phenotypes.

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