# 231. Improving lignocellulose saccharification by high throughput mutant screening and whole-genome sequencing of the mutants

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**Project Goals: We are using rice as the model to identify genes involved in grass cell wall biosynthesis and modification.**

To achieve cost efficient conversion of lignocellulosic biomass into biofuels, basic knowledge on the genes that control cell wall recalcitrance and enhanced saccharification is needed. We are using rice, a tractable model grass species, to identify genes involved cell wall biosynthesis and modification. We screened 5,000 rice fast neutron irradiated mutants (M2 lines) using a high throughput rice lignocellulose saccharification assay. From this screen, we identified 95 mutant candidates with altered saccharification efficiency. Of these, 45 mutant candidates showed increased saccharification efficiency and 50 mutant candidates showed reduced saccharification efficiency. Two of the mutants, were further characterized. The *rcs60* mutant segregated for reduced saccharification efficiency in the M2 generation. The *rcs60* progeny were dwarf and impaired in fertility. The *rcs815* mutant displayed 60% increase in saccharification.

We have initiated a whole genome sequencing project of 2,000 of the fast-neutron mutants in collaboration with the Joint Genome Institute (JGI). To date we have analyzed the whole- genome sequence for 45 lines and have detected 300 DNA changes. In the *rcs815* mutant, a single 13 bp homozygous deletion was detected. The deletion caused a frameshift mutation in the bZIP domain of a putative transcription factor that is conserved in switchgrass, sorghum and maize. This research provides an efficient approach to identify novel saccharification-related regulators in rice. The knowledge gained will be useful in improving other biofuel crops, including switchgrass, miscanthus and sorghum.

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