Development of a High-Throughput Genetic Transformation System for Switchgrass (*Panicum virgatum* L.)

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multitalented microbes for converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are using both testing and generation of large number of natural and modified plant samples as well as developing genomic tools for detailed studies into poorly understood cell wall biosynthesis pathways.

Switchgrass (*Panicum virgatum*), a native C4 perennial grass throughout North America, is an excellent candidate for the production of cellulosic biofuels. Genetic improvement of switchgrass through biotechnological approaches is expected to play a crucial role in modifying quality or quantity of biomass suitable for biofuel production. Development of genetic tools is essential for effective improvement of existing switchgrass cultivars. Switchgrass, like many other grasses, is generally considered difficult to genetically manipulate at the cellular level. The low transformation efficiency has been recognized as a bottleneck in genetic manipulation and functional test of transgenes in grasses. The establishment of a well defined, rapid and highly efficient genetic transformation system is an important prerequisite for genetic engineering of this species. We have successfully solved this bottleneck problem in switchgrass and established a high throughput system for the production of large numbers of transgenic plants.

By identification of highly tissue culture responsive genotypes and by optimization of transformation parameters, we have developed a highly efficient genetic transformation system for the widely used switchgrass cultivar Alamo. Embryogenic calli were induced from immature inflorescences or from seeds. Overexpression vectors and RNAi vectors were constructed and transferred into *Agrobacterium tumefaciens* strain EHA105 or AGL1. The hygromycin phosphotransferase (*hph*) gene was used as a selectable marker. After co-cultivation with *Agrobacterium*, the infected calli were transferred onto selection medium containing the antibiotic hygromycin. Resistant calli obtained after about six weeks of selection were transferred to regeneration medium. Regenerated green shoots were transferred to rooting medium, and the rooted plantlets were later transferred to the greenhouse. The timeline from callus infection to rooted plants was about 4 months. Regenerated plants were screened by PCR analysis. Stable integration of the transgenes into the plant genome was confirmed by Southern blot analysis. The transformation efficiency (number of independent transgenic plants/number of calli used for infection) reached more than 90%. Moreover, the system is consistent and highly reproducible. We easily produced more than 800 independent transgenic plants in 6 months. Our high throughput transformation system offers a solid basis for functional analysis of large numbers of genes in switchgrass.

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