191. 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 numbers of natural and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways.

Switchgrass (*Panicum virgatum* L.) is an outcrossing C4 perennial bunch grass native throughout North America. 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 bottle-neck 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 seeds or immature inflorescences. 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%. Since switchgrass is an outcrossing species, individual seeds within a cultivar may represent different genotypes. To solve this problem, we use inflorescences from a defined genotype to induce calli. Transgenic plants obtained from these calli have the same genetic background, allowing a precise comparison of the effects of transgenes. Importantly, our transformation system is

consistent and highly reproducible. We have produced transgenics from more than 70 gene constructs, with at least 30 independent transgenic events generated for each construct. This high throughput transformation system offers a solid basis for functional analysis of large numbers of genes in switchgrass.

To better characterize transgenic switchgrass plants, we proposed a standardized procedure for switchgrass sample collection by describing various developmental stages of switchgrass, defining the R1 stage as the stage at which tillers should be collected, and providing a description of how and what material should be analyzed. Such a standardized procedure will help to maintain consistency in switchgrass evaluation methods, enable comparisons of data obtained from different approaches and studies, and facilitate efforts towards improving switchgrass as a bioenergy crop.