DOE final report
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Since the last funding cycle we have made significant progress in creating new transgenic switchgrass plants that express *Corngrass*, the miR156 microRNA originally isolated from maize. These transgenic switchgrass plants provide a biomass that is easier and more efficiently degraded to simple sugars.

New transgenic *Cg1* switchgrass

Cg1 expression driven by the Ubiquitin promoter has a number of negative consequences in terms of root diameter, length and number (Fig. 1A). As outlined in Aim #1 of our renewal, we addressed this issue by re-designing the maize *Cg1* transgene to express the microRNA in aerial regions of the plant, but not in roots (Fig. 1B).

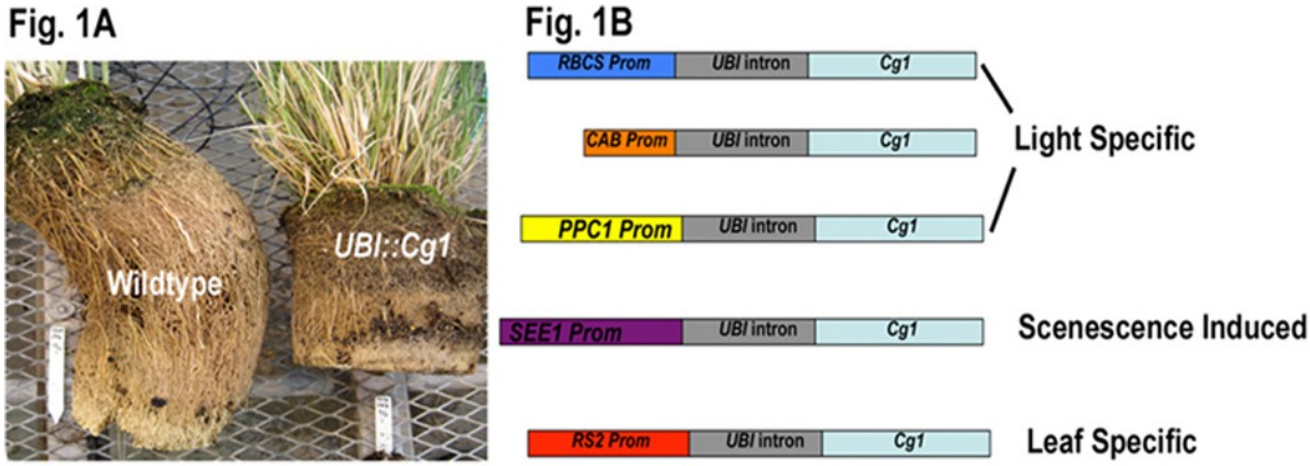

Figure 1. Addressing root *Cg1* problems. A) Phenotype of *UBI::Cg1* roots showing less root growth in the transgenic line. B) New *Cg1* constructs using aerial specific promoters.

New transformants expressing *Cg1* behind a variety of different light activated aerial promoters such as those for *RUBISCO* (*RBCS*), *CHLOROPHYLL A/B BINDING PROTEIN* (*CAB*) and *PHOSPHOPHOENOL PYRUVATE CARBOXYLASE* (*PPC1*) were generated. In addition, an aerial senescence promoter (*SEE1*) and a leaf specific promoter (*RS2*) were used (Fig. 1B). Nearly 100 switchgrass transformants were put into the field and observed for juvenile phenotypes. Apart from the *CAB* transformants whose roots resembled those of *UBI::Cg1*, all transgenic lines replicated the *UBI::Cg1* shoot phenotype but had healthier roots (Fig. 2). The majority of these new transformants have juvenile cell characteristics and have yet to flower after one year in the field and two years in the greenhouse. Thus, the new transgenics possess the characteristics of the previously described *UBI::Cg1* transgenics (Chuck et al. 2011), but with better root growth. These results show that promoter choice can have a large effect on plant development when over-expressing microRNA genes.

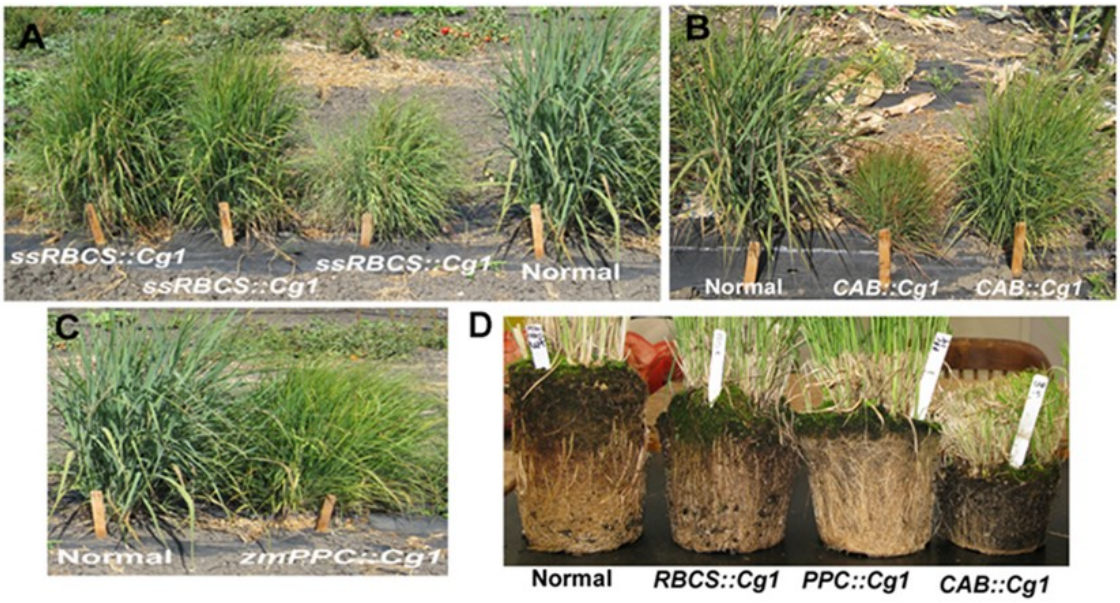

Figure 2. Field phenotypes of new *Cg1* transformants. A) *RBCS::Cg1*. B) *CAB::Cg1*. C) *PPC1::Cg1*. D) Comparison of root phenotypes. Except for the *CAB* lines, all transformants had healthier roots.

Saccharification assays of new *Cg1* biomass

After one year of growth in the field, biomass was harvested and subjected to saccharification assays after dilute base pre-treatment by the Lifeng Li in the Pauly lab. In order to access the increased starch levels caused by *Cg1* overexpression, assays were done utilizing alpha amylase added to the normal mix of hydrolytic enzymes and compared to assays without it.

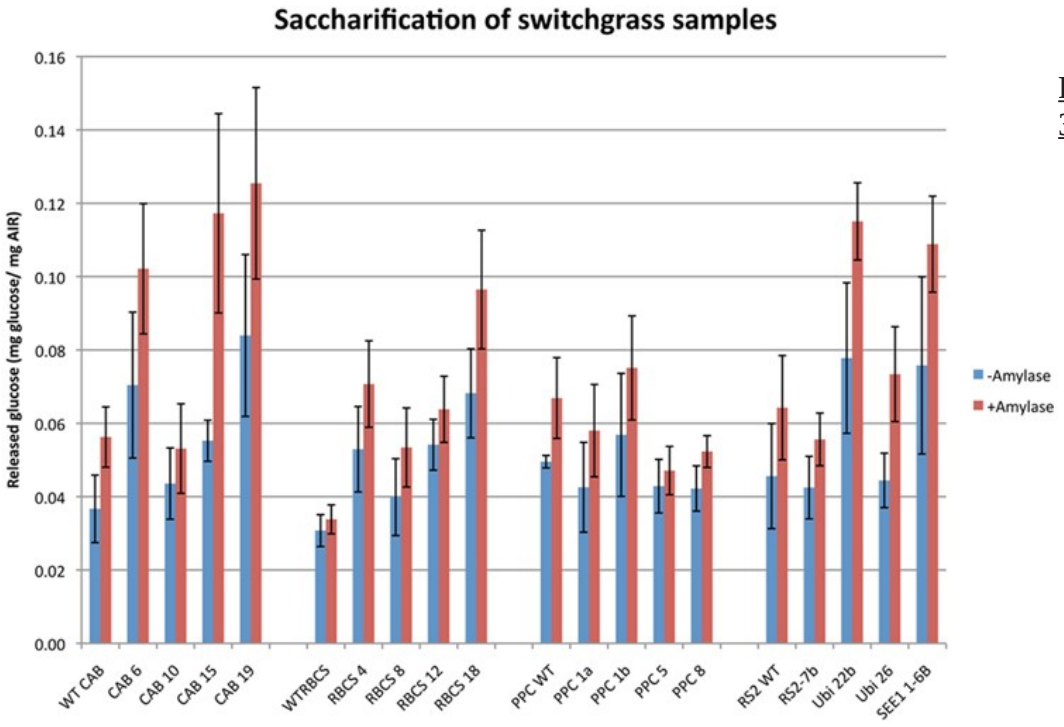

Figure 3.

Saccharification assays of new field grown *Cg1* biomass after dilute base pre-treatment with or without alpha amylase.

The saccharification data demonstrate that the *Cg1* transformants driven by the CAB, RBCS and SEE1 promoter have significantly elevated glucose release compared to wild type, similar to the UBI driven *Cg1* material. The PPC and RS2 promoters were not as effective in increasing glucose release. The addition of alpha amylase increased glucose release in the CAB, RBCS, SEE1 and UBI lines, and to a lesser degree, in wild type. The greatest increase was observed in the *CAB::Cg1* and *RBCS::Cg1* lines, both of which more than doubled the glucose release, similar to what was observed for *UBI::Cg1* lines (Fig. 3). Given these results in combination with the effects on the roots, we would chose the *RBCS::Cg1* lines as most likely to make switchgrass derived biofuels more economically feasible.

Analysis of knockouts of *Cg1* target genes

Reverse genetics were carried out with the Mutator system in collaboration with Pioneer/Dupont to determine the function of targets of the *Cg1* microRNA. Transposon insertions were obtained in the duplicated *Cg1* targeted *SBP* box transcription factors called *unbranched2 (ub2)* and *unbranched3 (ub3)*. Neither single mutant has a phenotype, however, double mutants display a strong decrease in the number of branches made by the tassel. An antibody was raised to the UB2 and UB3 proteins and used for immunolocalization. Interestingly, neither UB protein is found within the branch meristem despite being necessary for branch initiation. We hypothesize that *ub2* and *ub3* are functionally redundant factors necessary for controlling cell partitioning during branch meristem initiation. These results are currently being prepared for a manuscript to be submitted by the end of summer.

Publications:

Chuck, G., Brown, P., Meeley, R., and Hake, S**. (2014). The maize *SBP*-box transcription factors *unbranched2* and *unbranched3* affect yield traits by regulating the rate of lateral primordia initiation.** *Proc Natl Acad Sci***; 111(52):18775-80.**

Chuck, G., (2013). A high carb diet for biofuels. *Biofuels*

Chuck, G., Whipple, C., Jackson, D. and Hake, S. (2010). The maize *SBP*-box transcription factor *tasselsheath4* regulates bract development and establishment of meristem boundaries. ***Development*.** 137(8): 1243-50.