Pyramiding genes and alleles for improving energy cane biomass yield

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Project Goals: Our long term goal is to establish a new paradigm to accelerate energy cane breeding programs and maximize the biomass yield for biofuel production. Our specific objectives are: (1) Phenotyping extreme segregants of the F2 population for exploring the molecular basis of high biomass yield from transgressive segregation; (2) Mapping genes affecting biomass yield by transcriptome sequencing of the extreme segregants; (3) Identifying differentially expressed genes and alleles through analyses of transcriptomes of extreme segregants from the F2 population; (4) Developing gene- and allele-specific markers for implementation of marker-assisted selection in energy cane breeding programs.

As a C4 plant, sugarcane/energy cane has been recognized as one of the world's most efficient crops in converting solar energy into chemical energy. Traditional energy cane and sugarcane breeding via interspecific hybridization and backcrossing to *S. officinarum* improved stress tolerance and recovered high sugar and biomass yield. However, this approach reduced the genetic diversity of sugarcane and energy cane breeding materials and limited the potential maximizing biomass yield. We are developing a new paradigm to accelerate energy cane breeding programs and maximize the biomass yield for biofuel production.

The smallest genome in *Saccharum* is the *S. spontaneum* haploid AP85-441 developed by anther culture. It has a genome size of 3.4 Gb, 34% of a typical 10 Gb hybrid genome, and the smallest chromosome number at 2n = 4x = 32. Pacbio single-molecule real-time (SMRT) sequencing technology was used to sequence this haploid genome at 77X coverage. The *S. officinarum* LA Purple has 2n = 8x = 80 with genome size at 7.6 Gb. This genome was also sequenced at 80x using PacBio. Two F1 mapping populations were developed from crosses between double haploid AP83-108 and its pollen source SES208 and from S. *officinarum* LA Purple and *S. robustum* MOL5829. The individual genomes of these two F1 populations were sequenced at 5X coverage using Illumina HiSeq2500. The assembled draft genome of AP85-441 and LA Purple were used as references for SNP calling to map QTLs affecting biomass yield, and for gene expression analysis of extreme segregants.

An F_2 population with 2616 individuals was created from an interspecific cross between LA Purple x MOL5829 (2n = 80, x = 10). This population showed transgressive segregation with high yielding clones substantially exceeding the biomass yield of both parents. Field trial of the extreme segregants was carried out for three years in Hawaii. Biomass yield of the top 10 F_2 clones ranged from 71.5 to 122.1 MT/ha in 12 months, and the best performing clone showed 338% yield increase compared to its high yield parent LA Purple. The bottom 10 F2 clones had estimated biomass yield ranging from 3.4 to 8.2 MT/ha in 12 months, the worst performing clone showed yield decrease to only about 10% of LA Purple yield.

Leaf and stem internodes of high and low biomass F2 extreme segregants were used for RNA-seq to decipher the molecular mechanism of rapid plant growth and dry weight accumulation. Gene Ontology terms involved in cell wall metabolism and carbohydrate catabolism were enriched among 3,274 differentially expressed genes between high and low biomass groups. Specifically, up-regulation of cellulose metabolism, pectin degradation and lignin biosynthesis genes were observed in the high biomass group, in conjunction with higher transcript levels of callose metabolic genes and the cell wall loosening enzyme expansin. Furthermore, UDP-glucose biosynthesis and sucrose conversion genes were differentially expressed between the two groups. A positive correlation between stem glucose, but not sucrose, levels and dry weight was detected. We thus postulated that the high biomass sugarcane plants rapidly convert sucrose to UDP-glucose, which is the building block of cell wall polymers and callose, in order to maintain the rapid plant growth required for biomass accumulation. The gene interaction of cell wall metabolism, hexose allocation and cell division contributes to biomass yield, expanding our understanding at the molecular level required for energy cane breeding and engineering.