

The Center for Bioenergy Innovation (CBI)

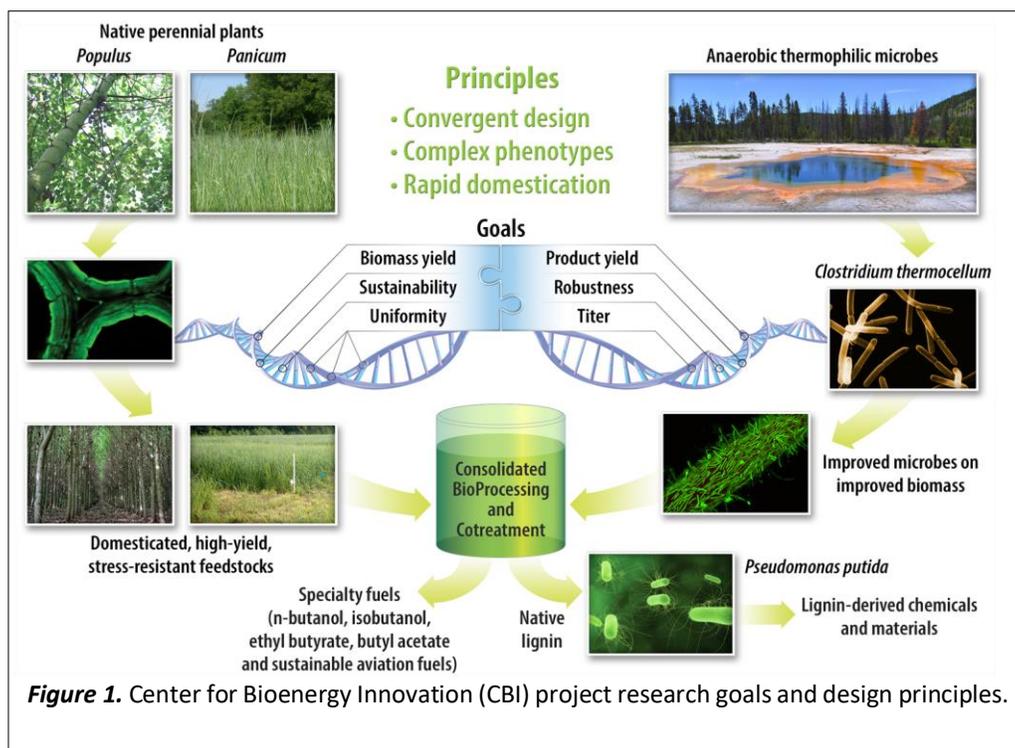
Performance Metric for FY20: Report on genomic science-based advances and testing of new plant feedstocks for bioenergy purposes.

Summary of progress on advances and testing of new plant feedstocks for bioenergy

September 2020

Introduction

The Center for Bioenergy Innovation (CBI) at Oak Ridge National Laboratory is a multidisciplinary research center with a vision to accelerate the domestication of bioenergy-relevant plants and microbes for an emerging bioeconomy (Figure 1). CBI has built on prior efforts in the BioEnergy Science Center (BESC). CBI has identified research targets to produce sustainable biomass feedstock crops using plant genomics and engineering and advance biomass processing based on consolidated bioprocessing and biological funneling. CBI’s ultimate aim is to produce valuable biofuels and bioproducts from plant derived feedstocks, polymers, and residuals.



This report highlights advances made by CBI and BESC from 2007-2020 and summarizes the material found in previous quarterly reports (<https://cbi.ornl.gov>) on:

1. The latest genomic insights into plant cell wall formation that informs the development of dedicated bioenergy crops (Q1 Metric Report). This report focuses on the relevant genomic insights due to CBI/BESC sustained efforts in poplar (*Populus* spp.). The development of genomic resources in poplar in conjunction with sustained common gardens of sequenced poplar genotypes has led to many genomic-based discoveries into plant cell wall formation. This has included a number of transcriptional factors for regulation of plant cell wall composition and structure. These plant-cell-wall genes have been shown to have functional homology beyond poplar into other plant species. This work has led to the development of improved genetic selection algorithms for improved breeding strategies.
2. The development of switchgrass variants as a dedicated bioenergy crop (Q2 Metric Report). CBI has generated a new collection of switchgrass lines with improved biomass properties using both targeted and non-targeted approaches. The collective efforts towards accelerating development of elite switchgrass lines resulted in creation of significant resources for the community, including the establishment of a high-efficiency switchgrass transformation system and vectors, a pipeline to streamline gene nomination, cloning and transformation, broad assessments of plant growth, and high-throughput approaches for characterizing chemical and physical properties of feedstocks as they relate to high-conversion efficiency.
3. The bioenergy-relevant insights gained from analyses of the poplar genome (Q3 Metric Report). The initial poplar genome sequence was published right before the launch of the DOE Bioenergy Research Centers (BRCs). Within the Bioenergy Science Center (BESC) and CBI, we have significantly expanded the poplar genomics resources by resequencing more than 1000 poplar genotypes, creating a poplar pangenome, and identifying centromeric regions overlooked by regular genome annotation. To facilitate the utilization of the large poplar genomics datasets, we have developed new computational biology capabilities for linking genomic variants to multiple plant traits, constructing coevolution networks, performing epistatic genome-wide association studies (GWAS), informing genomic selection via targeted quantitative trait nucleotide stacking, and unraveling complex gene-phenotype relationships using pleiotropy decomposition.
4. The results of field studies of modified switchgrass variants as dedicated bioenergy crops (Q4 Metric Report). Biomass yield is the single most important factor impacting economic sustainability of biofuels and bioproducts. Using transgenic, GWAS, familial crosses and quantitative trait loci (QTL) approaches, we have extensively examined how selected or modified switchgrass lines impact plant growth and ecosystem function. Further, our

switchgrass GWAS fields provided information on genetic variation in switchgrass and enabled the identification of genotypes that have high and uniform biomass yield across diverse environments. GWAS analyses also enabled identification of genetic loci associated with high biomass yield and sustainability, with subsequent integration into genomic selection models. Further, QTL and association mapping enabled CBI researchers in rapid gene discovery. Consistently, we identified QTL for phenotypic traits and metabolites in an F₂ switchgrass validation population.

Below we provide summaries of the four subject reports. More details are contained within the full reports.

A. The latest genomic insights into plant cell wall formation that informs the development of dedicated bioenergy crops.

The CBI and BESC developed a multi-faceted toolkit of QTL, structured pedigrees, and GWAS studies of the bioenergy feedstock *Populus*. Using these tools, we focused on understanding plant cell wall formation and enhanced productivity of bioenergy plantations across a wide range of environments, ensuring a stable supply of feedstock for lignocellulosic biofuel refineries. Key results from these studies demonstrated that:

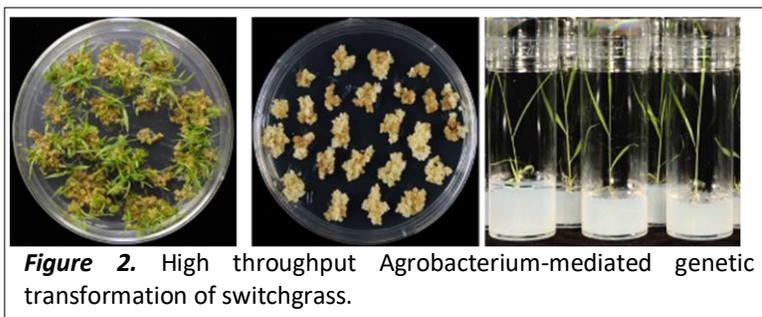
- 1) Biomass related traits, such as cell wall chemistries, were genetically controlled and could be manipulated via genetic transformation or genomic selection approaches.
- 2) We could accurately estimate quantitative components of phenotypic variation and ultimately identify the underlying loci controlling traits of interest using a network of geographically distributed *Populus* GWAS plantations.
- 3) We can predict cell wall chemistry traits using high-throughput phenotyping assays, allowing the assessment of thousands of biomass samples. Further, we were able to link variation at the phenotypic level to specific changes in DNA structure, such as single nucleotide polymorphisms and single amino acid substitutions in functioning proteins using a combination of the genome-wide association population and DNA marker libraries
- 4) We now understand the mechanisms leading to phenotypic change, and we identified causal substitutions at the amino acid level.
- 5) Finally, we developed a series of genomic selection algorithms that predict progeny performance of selected crosses in *P. trichocarpa*. These algorithms are based on and target increased biomass production, improved cell wall chemistry traits and enhanced disease resistance. These crosses have been made and progeny are now currently growing in field trials and have completed their first year of growth. We will not know

that final outcome and success of this prediction until after the end of the fourth growing season (approximately Fall 2022).

B. The development of switchgrass variants as a dedicated bioenergy crop.

The Center for Bioenergy Innovation is focused to develop *Panicum virgatum*, switchgrass, as a bioenergy crop using GWAS and QTL mapping populations, genetic tools, and an assembled and annotated reference genome. Switchgrass is a perennial, fast growing, warm season grass, which is widely adapted to native environments across North America, making switchgrass well suited to development in target marginal environments. Switchgrass is largely an obligate outcrossing species thus self-pollination is not feasible with this species. Over the last three years, significant advances were made in understanding, manipulating, and managing switchgrass biomass and plant cell wall traits and their relation to conversion efficiency. Researchers showed that multiple plant genes control cell wall recalcitrance, and that manipulation of these genes can yield lower recalcitrance perennial feedstocks. Key results from these studies demonstrate:

- 1) We have developed a CRISPR-Cas9 system for switchgrass which increased the overall efficiency of the switchgrass transformation from 5% up to 90% and decreased the turnaround time to less than four months (Figure 2; Mann et al., 2012; Nageswara et al., 2013).

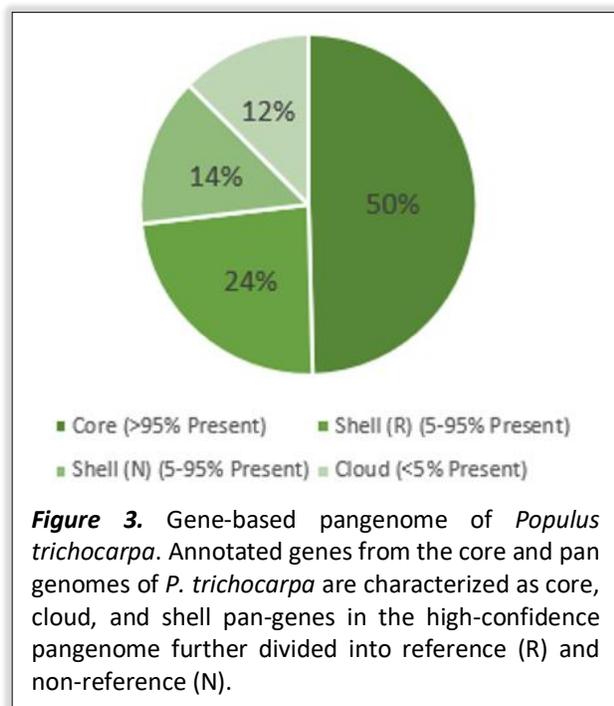


- 2) We increased the visualization of the chemical nature of pretreated and deconstructed biomass surface at the submicron level using imaging techniques based on atomic force microscopy (Raman spectroscopy) and mass spectrometry.
- 3) We used our switchgrass transformation pipeline to evaluate lignin and pectin synthesis pathway genes (*PvGAUT4-KD* and *PvGAUT1-KD*), cellulose biosynthesis genes (*PvCesA4* and *PvCesa6* RNAi lines), hemicellulose biosynthesis gene (*PvIRX10-KD*) and hormone metabolism (*PvGA2Ox-OE*) pathways as well. Transgenic switchgrass *PvCesa6*-overexpressing lines had reduced lignin content, increased sugar release efficiency, but reduced overall biomass productivity (Mazarei et al., 2018).
- 4) We have focused studies on enhanced biomass productivity under marginal conditions using genomic selection of switchgrass, managed microbiomes, and enhancement of feedstock performance with a new biocatalyst.

C. The bioenergy-relevant insights gained from analyses of the poplar genome.

Poplar (*Populus* spp.) is one of the two primary plant feedstocks that have been studied in the BESC project (2007-2017) and CBI (2018-present). Over the past 12 years, we have sought and continue to accelerate domestication of poplar plants for bioenergy production. The genome of *P. trichocarpa* was the 3rd plant genome sequenced in 2006 (Tuskan et al., 2006). With the leadership of Oak Ridge National Laboratory and the DOE Joint Genome Institute, rich genomics resources have been created for poplar, including an improved high-quality genome sequence assembly and gene annotation, genome-resequencing data for more than 1000 poplar genotypes, and poplar gene expression atlas for various tissue types and experimental conditions. Further, the poplar genomics resources have been widely used by a large scientific community including the researchers in BESC and CBI for identification of genes associated with bioenergy traits. Key results from studies using the poplar genome are:

- 1) We created a new SNP dataset that includes genetic variations found in 882 poplar trees and provides useful information to scientists studying plants as well as researchers more generally in the fields of biofuels, materials science, and secondary plant compounds.



- 2) Using a population genomics approach, we have determined the distribution of neutral and adaptive genetic variation in a *Populus trichocarpa* association study population consisting of 1,100 trees collected from across the range of the species from California to British Columbia.

- 3) We constructed the core and pan genomes of *P. trichocarpa* from 1,036 genotypes to exploit the natural variation of this population for identifying potential biologically relevant gene targets (Figure 3).

- 4) We were awarded the 2018 Gordon Bell Prize by achieving the fastest scientific calculation using a custom correlation coefficient (CCC) approach that was implemented on the Summit supercomputer. CCC can identify groups of SNPs that tend to co-

occur in a population and consequently can be used to find combinations of SNPs that associate with specific phenotypes.

- 5) We identified genes in the *P. trichocarpa* pan-genome, which affect poplar leaf cuticular wax, using nature's knockout association test. Using genome-wide eQTN mapping, we predicted regulatory elements for more than 20,000 genes and identified transcriptional master regulators regulating more than 100 bioenergy-relevant genes.
- 6) We discovered a susceptibility-associated locus that was predicted to encode a putative G-type D-mannose-binding receptor-like kinase. Multiple lines of evidence, including allele analysis, transcriptomics, binding assays, and overexpression, support the hypothesized function of these candidate genes in the *P. trichocarpa* response to pathogen *S. musiva* (Muchero et al., 2018). Furthermore, we found that a G-type lectin receptor-like kinases (LecRLKs) in *P. trichocarpa* mediates the interaction between poplar and fungus *Laccaria bicolor* (Labbé et al., 2019).

D. The results of field studies of modified switchgrass variants as dedicated bioenergy crops.

Switchgrass is the second of the two primary plant feedstocks that have been studied in BESC and CBI. Switchgrass is a native perennial C4 grass that is considered a leading candidate for a dedicated lignocellulosic biofuel feedstock owing to its high biomass production, wide adaptation to various geographic and temperate climates, and low agronomic input requirements (van der Weijde et al., 2013). One of the major problems with lignocellulosic crops is the resistance of the cell wall to chemical, microbial, or enzymatic deconstruction into fermentable sugars, a phenomenon known as cell wall recalcitrance (Himmel and Bayer, 2009). Reducing the recalcitrance of bioenergy feedstocks was a primary goal of the BESC project by genetically modifying switchgrass cell wall characteristics. While these modifications were successful, they can often have unintended consequences when these transgenic lines are grown in the field. Therefore, a variety of BESC/CBI switchgrass field experiments have been conducted to evaluate how variation in switchgrass cell wall chemistry alters switchgrass growth, productivity, disease resistance, and sustainability. Diverse switchgrass lines, including those involving transgenic materials, natural variants, and genome wide association studies and F₂ populations have been examined in field sites across multiple growing seasons and multiple sites. Key results from these studies highlight:

- 1) Transgenic switchgrass lines with altered lignin biosynthesis (Baxter et al., 2014, 2016) had equivalent biomass yield relative to the wild-type and empty vector control lines when grown in the field (Figure 4). Further, sugar release was improved by up to 34% and ethanol yield by up to 28% in the transgenic lines relative to controls.

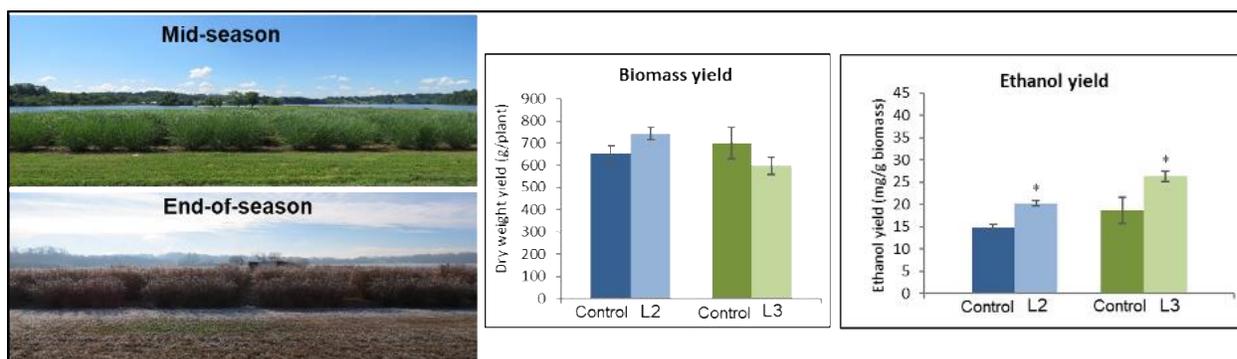


Figure 4. The 2013 field trials of reduced recalcitrance COMT-KD switchgrass planting at the UTK (Knoxville, TN) field site. Bars with asterisk are significantly different from controls at $p \leq 0.05$ calculated using *t*-tests for pairwise comparison with SAS software.

- 2) Hybridization of transgenic low recalcitrant switchgrass lines with a high-yielding locally selected genotype resulted in improved agronomic performance and enhanced biomass quality in the offspring.
- 3) Over the course of five growing seasons, we found no impact of growing transgenic lines in the field on soil pH or the total concentrations of 19 soil-based elements. In addition, there were no detectable effects of the transgenic plants on bacterial diversity, richness, or community composition. We detected no changes in the capacity for soil carbon storage (Chauhan et al., 2014; DeBruyn et al., 2017).
- 4) Switchgrass mapping projects were initiated to identify genetic loci associated with important traits and to initiate a marker-assisted breeding program. A total of 352 genotypes of the switchgrass association mapping population were evaluated in two locations for three years. From this set, 20,233 single nucleotide polymorphisms were obtained following a genotyping by sequencing protocol of the population. More than 500 SNPs were found associated with important biomass traits. Six genotypes were identified from switchgrass natural variants with superior sugar release, reduced lignin content and high biomass yield across multi-environments.
- 5) Switchgrass field experiments of a GWAS panel consisting of up to 400 diverse accessions are being performed to enable rapid domestication and increased sustainability in switchgrass. Three switchgrass GWAS fields have been established at: UGA (Tifton, GA), UTK (Knoxville, TN), and UGA (Watkinsville, GA) field sites.

- 6) By coupling DayCent simulations of crop growth and carbon cycling with conversion technologies, we showed current-day cellulosic ethanol production from switchgrass is equal to reforestation and greater than grassland restoration for greenhouse gas mitigation.

E. Conclusions

Over the past dozen years, CBI/BESC has made significant strides in accelerating the domestication of poplar and switchgrass to enable a thriving bioeconomy. We have made significant progress in understanding, manipulating, and managing these two biomass feedstocks. Studies conducted over the last three years provide new insights into the molecular mechanisms underlying biomass recalcitrance, disease resistance, and tolerance to abiotic stress. Further, we have established unique plant, microbial, and genomic resources paired with advanced computational capabilities which lay the foundation for future research that will maximize the potential of poplar and switchgrass for bioenergy production.

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