Application of Base Editing Technology in Poplar

Guoliang Yuan*[1] (mailto:yuang@ornl.gov), Haiwei Lu[2], Md. Mahmudul Hassan[2], Tao Yao[1], Wellington Muchero[1], Jin-Gui Chen[1], Xiaohan Yang[1], and **Gerald A. Tuskan**[1]

[1]Center for Bioenergy Innovation, Oak Ridge National Laboratory; [2]Biosciences Division, Oak Ridge National Laboratory

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The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* **CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by** *in planta* **modifications and biological funneling of lignin to value-added chemicals.**

Poplar (*Populus* spp.) is an important perennial woody bioenergy crop. In CBI, we seek to develop new capabilities for genetic improvement of poplar as a sustainable source of biomass for biofuels production. To achieve this goal, a host of new technologies are indispensable for accelerating the domestication of poplar plants. Base editing that directly generates precise point mutations is one of the most recent advances in the area of genome engineering. Although base editing has been used in multiple plant species, its application in poplar has not been well established yet. Here, we applied base editing technology in hybrid poplar clone 717 (*P. tremula* × *P. alba*) and in *P. deltoides* WV94, aiming to establish a robust system for the high-precision genome engineering in poplar. Our previous genome-wide association study (GWAS) and RNA-seq in poplar identified one transcription factor that was associated with disease resistance (Muchero et al., 2018). Another transcription factor was recognized as a key regulator in response to heat stress in plants but it's role in poplar has not been studied yet (Charng et al., 2007). To validate the functions of these two transcription factors, we generated loss-of-function mutants in these poplar lines by introducing pre-mature stop codons in the protein-coding sequences using a base editor. So far, the desired point mutation has been detected in genetically modified hybrid poplar 717 plants, indicating that the base editor functioned well in that poplar line. Also, base-editing constructs have been engineered into *P. deltoides* WV94, and transgenic plants are being characterized for desired point mutations in the target genes. The impact of the mutations mediated by base-editing on phenotypic

traits (e.g., disease resistance, heat tolerance) will be evaluated in the genetically-modified poplar plants in the future.

References

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