## A Sorghum NAC Gene Affects Vascular Development and Biomass Properties

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Project Goals: This project, "Coordinated Genetic Improvement of Bioenergy Sorghum for Compositional and Agronomic Traits", is focused on the identification of natural variants that increase net energy production from bioenergy sorghum. Since increased digestibility is often associated with decreased agronomic performance, these traits need to be considered together. Our team includes biochemists at Berkeley performing highthroughput compositional analysis of sorghum biomass, and geneticists at Illinois running field experiments on large panels of diverse photoperiod-sensitive sorghum accessions. Detailed characterization of natural variants that modulate biomass yield and composition, such as the NAC074 variant presented here, will help guide the engineering of nextgeneration grass feedstocks.

Sorghum bicolor is a C4 grass widely cultivated for grain, forage, sugar and biomass. The sorghum Dry Stalk (D) locus controls a qualitative difference between juicy green (dd) and dry white (D-) stalks and midribs, and co-localizes with a quantitative trait locus for sugar yield. Here, we apply fine-mapping and GWAS to identify a premature stop codon in a NAC gene as the candidate polymorphism underlying the sorghum D locus. Allelic variation at D affects grain and sugar yield, biomass composition, and vascular anatomy in nearly-isogenic lines. Green midrib (*dd*) NILs show reductions in lignin and the number of vascular bundles in stalk tissue, but produce higher sugar and grain yields under well-watered field conditions. Increased yield potential in *dd* NILs is associated with increased stalk mass and moisture, higher biomass digestibility, and an extended period of grain filling. Transcriptome profiling of midrib tissue at the 4-6 leaf stages, when NILs first become phenotypically distinct, reveals that dd NILs have increased expression of a miniature zinc finger (MIF) gene. MIF genes dimerize with and suppress zinc finger homeodomain (ZF-HD) transcription factors, and a ZF-HD gene is associated with midrib color variation in a GWAS analysis across 1694 diverse sorghum inbreds. Together, these results suggest that interaction between NAC, MIF, and MIF-targeted ZF-HD transcription factors regulates vascular development and agronomic potential in cereals.

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