

Quantitative trait loci analysis of leaf carbon isotopic composition in the C₄ grass *Setaria*

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Project Goals: Genetically tractable model systems closely related to bioenergy grasses need to be developed to drive the crop improvement required for large scale, ecologically sustainable bioenergy production. *Setaria viridis* is an ideal candidate C₄ panacoid grass. The objectives of this project are to utilize genomic, computational and engineering tools to begin the genetic dissection of drought and density response in *S. viridis*. This will be achieved through: 1) Quantitative trait and association genetics; 2) novel controlled environment and field phenotyping combined with molecular and chemical profiling; 3) development of metabolic and gene networks; 4) development of transformation technologies; 5) reverse genetic testing of candidate genes.

Abstract: Leaf carbon isotopic composition ($\delta^{13}\text{C}_{\text{leaf}}$) is a proxy for water use efficiency (WUE) in high throughput phenotyping. A population of 217 recombinant inbred lines (RIL) derived from the C₄ parental lines of A-10 (*Setaria viridis*) and B-100 (*Setaria italica*) was used to identify quantitative trait loci (QTL) controlling $\delta^{13}\text{C}_{\text{leaf}}$. To test the variation in WUE in this population, the RILs were grown under drought and density field experiments conducted at the University of Illinois Champaign-Urbana in 2013 and 2014. The leaf $\delta^{13}\text{C}$ values were significantly more negative in the drought and low-density treatments in both 2013 and 2014 ($P < 0.0001$). Additionally, phenotypic correlation analysis of growth and leaf traits found that $\delta^{13}\text{C}$ values were most highly correlated with leaf N content/C:N ratio in both experiments and years (mean $r = -0.42$; $P < 0.0001$). Significantly QTLs were found for $\delta^{13}\text{C}_{\text{leaf}}$ at potentially 11 locations on the genome. The two QTLs with the largest additive effects were on chromosome 5 and 8 and were present in both the drought and density experiments in both years, contributing $0.28 \pm 0.04\text{‰}$ and $0.19 \pm 0.04\text{‰}$ to $\delta^{13}\text{C}_{\text{leaf}}$ values, respectively. These two QTLs were also found for leaf C:N ratio. The QTL on chromosome 5 is a pleiotropic

locus identified for several growth-related traits in the field experiments, but the QTL on chromosome 8 may be more specific to WUE. Having identified QTLs for $\delta^{13}\text{C}_{\text{leaf}}$ that are consistent across experiments, we move closer to using marker-assisted approaches to breed for WUE in C_4 plants.

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