Comparative metabolic network analysis of *Setaria italica* and *Setaria viridis*

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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; and 5) reverse genetic testing of candidate genes.

To achieve these goals, we created genome-scale metabolic network reconstructions of *Setaria italica*, one of the oldest crops in the world, and *Setaria viridis*, a wild ancestor. Comparative network analysis will shed light on how these grasses are able to allocate metabolic resources for biomass production and C₄ photosynthesis, often limited during abiotically stressed conditions. We were motivated by the question of whether the process of domestication of *S. italica* over millennia might have had an effect on the overall metabolic network function. We analyzed both network reconstructions using Flux Balance Analysis (FBA), a computational technique that generates steady-state flux profiles of an organism’s reaction network while optimizing for the production of growth-related metabolites. The use of FBA-based computational modeling allowed us to compare key aspects of primary and secondary metabolism between *S. italica* and *S. viridis*. We evaluated overall metabolic network function in both species under drought and nutrient-limiting conditions in the following specific ways: (a) we probed for differences in metabolic traits relevant to biomass such as production of carbohydrates, synthesis of amino acids, and ATP requirements in water or nutrient replete and limiting conditions; (b) we assessed network robustness by systematically removing individual genes and setting any associated reaction fluxes to zero, and subsequently evaluating for the production of biomass; (c) we searched for dependencies between reactions and identified the presence of correlated reaction sets (reaction modules that tend to function together). Future work will involve integrating gene expression datasets with our FBA models in order to generate condition-specific metabolic networks.

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