## 25. Geminivirus replicons for targeted modification of cereal genomes

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http://sviridis.org/
http://foxmillet.org/

**Project Goals**: "A Systems-Level Analysis of Drought and Density Response in the Model C4 Grass Setaria viridis". The specific aims of the proposal are to: 1) Identify QTL for the effect of drought and density on biomass and seed yield components of Setaria. 2) Conduct in–depth physiological profiles in roots and leaves of a subset of selected lines 3) Integrate datasets and develop metabolic and gene networks for Setaria 4) Develop transformation technologies for Setaria viridis 5) Functionally examine the role of candidate genes deduced by network models; and 6) Develop protocols and best practices for monitoring gene flow in transgenic Setaria. To achieve these aims we will produce one of the most extensive molecular characterizations of plant growth in the field to date, generating several million data points that will be collected from physiological and molecular genetic studies. We will develop novel informatics models and network tools that will guide future molecular characterization in S. viridis and guide breeding efforts in major feedstock targets.

We developed a deconstructed version of the wheat dwarf virus (WDV) for use as a delivery vector for sequence-specific nucleases and DNA donor templates to enable efficient genome engineering of cereal species. These deconstructed WDV vectors lack the movement and coat protein coding sequences, and consequently cannot spread from cell-to-cell or to other plants. The WDV vectors replicate in transformed wheat scutella, calli, and protoplasts, leading to up to a 110-fold increase in the expression of a GFP reporter gene compared to the non-replicating control. CRISPR/Cas9 sequence-specific nucleases were cloned into the WDV vectors. The increased copy number of the nucleases coding sequences and the donor template led to enhanced levels of gene targeting efficiencies in wheat cells. Other important crops such as corn and rice, as well as green millet, were also found to be suitable hosts of the WDV replicon. The results demonstrate a new platform for genome engineering of cereal crops.

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