Expanding the breeder's toolbox for perennial grasses: Sequencing the genome of *Brachypodium sylvaticum* to establish a tractable perennial grass model system and the identification of transgenes conferring tolerance to abiotic stress.

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Project Goals: The project aims at using a systems-based approach to develop new breeding tools for perennial grasses using the perennial grass *Brachypodium Sylvaticum* as a model, and apply these tools towards the improvement of switchgrass (*Panicum virgatum* L.). The objectives of the research project are: 1. Develop a CENH3-based method for creating doubled haploid grasses and demonstrate it in switchgrass; 2. Use the model perennial grass *Brachypodium sylvaticum* to identify combinations of transgenes that confer tolerance to multiple abiotic stresses; 3. Develop a gene containment system to minimize gene flow from transgenic switchgrass; 4. Genome sequencing of *B. Sylvaticum*

Most of our knowledge of grass biology and responses to abiotic stress is based on studies of annual grasses like rice, wheat and barley. However, most of the grasses being developed as biomass crops are perennial. Annuals and perennials differ in many important physiological and developmental aspects, some of which that may be particularly relevant to stress tolerance. Unfortunately, the perennial grasses being developed as biomass crops (e.g. switchgrass and Miscanthus) are difficult experimental subjects due to their large size, long generation time, complicated genetics and large genomes. Thus, there is a pressing need for a tractable perennial grass model to study areas like abiotic stress tolerance and, in particular, to rapidly test transgenic approaches before moving into biomass crops.

We previously established key tools that allow *Brachypodium sylvaticum* to be used as perennial model grass including a highly efficient transformation protocol and inbred lines. Missing from this list is a high quality genome sequence, a prerequisite for a modern model organism. To fill this need, we sequenced the genome of *B. sylvaticum* to ~80x depth using PacBio long-read technology. The current assembly contains 358Mb of sequence in 1,118 contigs with a N50 contig length of 874Kb. The contigs are being ordered and orientated into high quality chromosome-level assemblies using a high-density genetic map created from 288 F2 individuals. A deep RNA-Seq expression atlas of 16 different tissues and developmental stages will aid gene annotation of the chromosomal-level assembly. A replicated set of another 92 samples, tested under a variety of abiotic stresses, is also being probed by RNA-Seq to identify stress responsive genes and their associated gene regulatory networks in this model perennial grass. Matched RNA-Seq samples have already been generated for the small annual grass, *Brachypodium distachyon,* allowing a unique comparison of stress response between closely related annual and perennial species.

We completed the transformation of *B. Sylvaticum* with constructs containing 33 genes shown to be associated with enhanced abiotic stress tolerance and source-sink relationships in monocots. Single and/or combination of these genes were overexpressed using either constitutive or stress inducible promoter. We have generated 5-7 single copy insert homozygous T2 lines for all constructs. The response of those transgenes lines to water deficit stress, salinity and a combination of stresses is being tested. Preliminary results revealed a higher biomass in the transgenic plants grown under stress. Our research indicates a great potential for the development of grasses with improved performance and yield under abiotic stress.