

200. Global Analysis of Alternative Splicing and Epigenetic Regulation of Gene Expression in Response to Drought Stress in Sorghum

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Abiotic stresses including drought are major limiting factors of crop yields and cause significant crop losses. Acquisition of stress tolerance to abiotic stresses requires coordinated regulation of a multitude of biochemical and physiological changes, and most of these changes depend on alterations in gene expression. The major goal of this proposal is to investigate drought-induced global changes in alternative splicing events and epigenetic modifications that are important for drought tolerance in Sorghum. The specific objectives of this project are: 1) Perform global analysis of differential regulation of expression and alternative splicing of primary transcripts from both protein coding and non-coding genes in drought sensitive and tolerant cultivars. 2) Perform global analysis of accessible chromatin landscape changes in drought sensitive and tolerant cultivars in the presence and absence of drought stress. 3) Analyze genome-wide changes in histone modifications in response to drought stress in drought sensitive and tolerant cultivars in the presence and absence of drought stress. The focus of this objective will be on global analysis of stress-induced changes in histone modifications. Our ultimate goal is to correlate chromatin modifications to changes in gene expression and alternative splicing, and to identify genes and regulated splicing events that play a role in drought tolerance.

To identify two drought tolerant and two susceptible cultivars of sorghum for our proposed experiments we screened seven sorghum varieties, which include the sequenced BTx623 cultivar. Based on the growth phenotype we selected two drought tolerant and two drought susceptible cultivars. To investigate differential regulation of gene expression and alternative splicing in response to drought we performed RNA-Seq for all four lines in control and drought-treated seedlings. Two biological replicates were used for each sample. Prior to RNA-Seq we confirmed the efficacy of drought treatment by testing the expression of known drought-responsive marker genes using either semi-quantitative PCR or real-time PCR (qPCR). Analysis of RNA-Seq data has revealed that between 1300 and 2000 genes are differentially expressed in response to treatment and a large majority were up-regulated. A large fraction of the up-regulated genes were common among all four cultivars. However, each cultivar had a unique set of up-regulated genes in response to drought treatment. Selected candidate genes that are differentially expressed or alternatively spliced were confirmed using semi-quantitative and quantitative RT-PCR. To study differential alternative splicing we developed a tool called iDiffIR that detects statistically significant differences in intron retention across conditions. Preliminary analysis using this tool has detected hundreds of differential intron retention events in response to drought treatment, indicating that splicing is involved in the response to drought.

As the Sorghum transcriptome is not very well annotated, with very few known splice variants per gene, we sequenced the transcriptome of one cultivar (BTx623) using the Pacific Biosciences Iso-Seq platform that provides long reads and permits accurate prediction of splice variants. This is a new technology with very limited tools for the analysis of such data, especially due to the high error rate of the resulting reads. We developed a new pipeline called TAPIS (Transcriptome Analysis Pipeline for Isoform Sequencing) that predicts both isoforms and alternative polyadenylation sites and uses either the reference genome or Illumina reads for error correction. Out of 33,032 genes in version 2 of the Sorghum annotations, we found 7,108 genes with one or more reads covering both first and last exons. Analysis of this data revealed >2000 novel alternative splicing events, more than doubling the number of known intron

retention events. We also uncovered over 600 loci that were misannotated and evidence for around 500 novel genes, some of which are similar to known protein-coding genes in other plants. As the 3' ends of transcripts are well-covered by Iso-Seq reads, we were also able to uncover the landscape of polyadenylation sites. Transcripts from over 2000 genes were found to have two or more polyadenylation sites. Of these, around 4% were in the coding region or the 5' UTR.

Intron retention is the predominant form of alternative splicing in plants. However, very little is known about the regulatory sequences in pre-mRNAs that regulate this event. To identify putative elements that contribute to intron excision or retention, we have developed a computational pipeline that uses all known IR and intron excision events in Sorghum and other land plants to detect over-represented hexamers that are conserved across species. We have found over-represented hexamers that fall either in the flanking exons, or in the intron itself. A large fraction of these hexamers are also known to regulate splicing in animals. In view of their conservation and similarity to known splicing regulatory elements in animals, the elements we detected are likely to be functional.

In our proposal, we suggested to use DNAaseI-Seq to analyze open chromatin. However, a new method called DNase-FLASH (DNase I–released fragment-length analysis of hypersensitivity) that is superior to DANseI-Seq was reported recently (Vierstra et al., *Nature Methods* 11, 66–72, 2014). We have been standardizing this method for Sorghum. We anticipate sending DNA samples from DNase-FLASH and chromatin immunoprecipitation in the near future.

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0010733