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PNNL-30581

High-throughput screening of Protein-DNA interactions in Sorghum

September 2020

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Report

Background and Significance

Sorghum is a widely used agricultural staple, as well as an attractive bioenergy crop. Des the use of homology modeling, the function of 8,683 Sorghum genes (25% total genome) remain annotated as “proteins of unknown function” (PUFs). Transcriptome profiling experiments have demonstrated that PUFs constitute 32% of Sorghum transcripts that are differentially expressed upon treatment by various stresses. A similar report showed that PUFs constitute 15% of the top 100 differentially regulated Sorghum genes during drought. These findings strongly suggest the need for characterizing PUFs and obtaining a detailed molecular level understanding of Sorghum’s unique ability to cope with abiotic stress. These findings can present a valuable opportunity for crop improvement in future crop engineering efforts. Like most plants, Sorghum dedicates a large portion of its genome (>5%) to genes predicted to code for Transcription factors (TFs). We need a high-throughput method to systematically screen the proteome for all proteins that have similar functional attributes, such as the capacity to bind DNA. Furthermore, we need a high-throughput method to identify the specific sequences to which each DNA-binding protein binds.

Specific aims

Drought is the largest stress affecting agricultural crops, resulting in substantial reductions in yield. Plant adaptation to water stress is a complex trait involving changes in hormone signaling, physiology, and morphology. Sorghum bicolor L. Moench is a C4 cereal grass, agricultural staple, and is particularly drought tolerant. We aim to obtain a mechanistic understanding of the proteome level changes resulting in Sorghum drought tolerance.

Aim 1. Nuclear protein profiling to identify proteins associated with drought adaptation

Aim 2. Develop a high-throughput method to assign DNA-binding function to uncharacterized proteins

Results

Protein profiles are enriched for organelle and cytoplasmic compartments

We used two sorghum genotypes with contrasting drought adaptation phenotypes. Genotype RTx430 is pre-flowering drought tolerance and BTx642 is pre-flowering drought susceptible. To identify the potential mechanisms by which RTx430 uniquely adapts to water deficit, we compared the RTx430 and BTx642 organelle and cytoplasmic protein profiles after 8 weeks of drought stress. In total, our analysis resulted in confident identification of 3,683 and 4,130 proteins in cytoplasmic- and organelle-enriched samples, respectively. A total of 3,103 proteins were identified in both sample types. To evaluate whether our procedure accurately enriched for organelle and cytoplasmic protein profiles, we first compared the abundance of histone, chloroplast, and cytosolic ribosomal proteins independent of drought stress (Fig. 1). We observed cytosolic ribosomal proteins, including ribosomal L13, S16, and L20 proteins were more abundant in cytosolic protein samples (Fig. 1A). Conversely, chloroplast and histone proteins were predominately more abundant in organelle enriched samples (Fig. 1B, C). These data suggest a successful enrichment for protein profiles representative of the different cellular compartments.

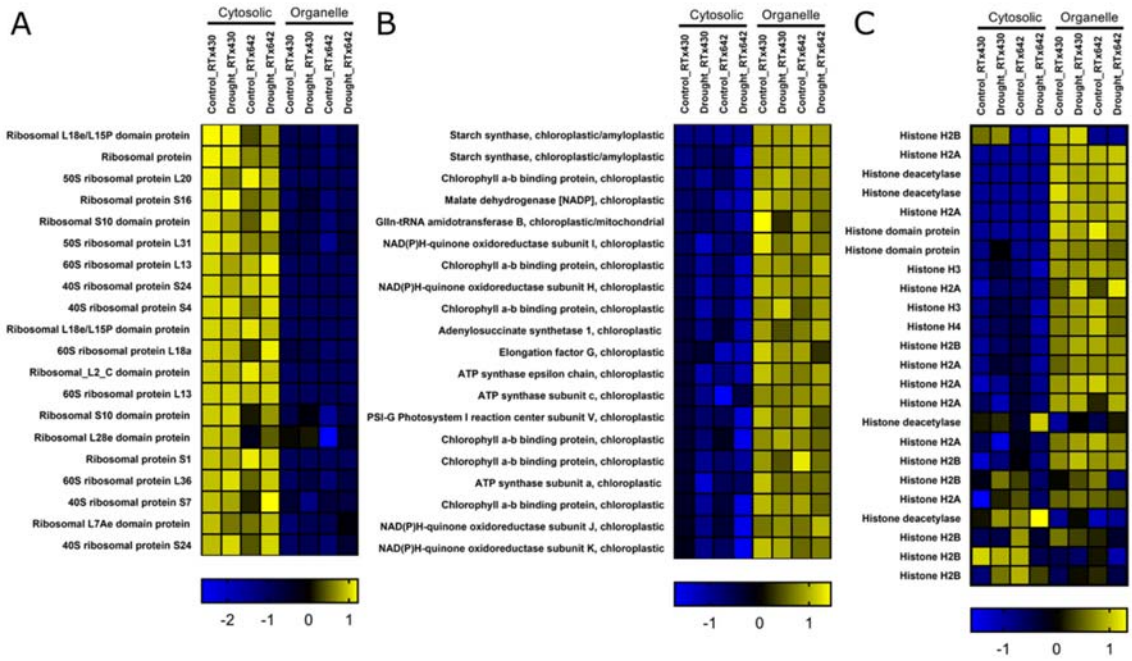

Figure 1. Evaluation of cytosolic and organelle enrichment. Ribosomal proteins abundant in the cytoplasm were more abundant in cytosolic-enriched samples (A), while photosynthesis related chloroplastic (B) and nuclear histone proteins (C) were more abundant in organelle enriched samples. Color and scale bars represent z-score transformed protein abundances.

Protein profiles in both cellular compartments are impacted by drought

A principal component analysis was performed to understand the drought impact at the organelle and genotype level. PC1 explains 36.8% of variation for organelle-enriched samples, which separated the treatment groups and PC2 explains 21.9% of data variation that accounts for the differences between genotypes. Similarly, 25.9% of data variation is explained in PC1 for the cytosolic fraction which is a result of the drought. Unlike organelle-enriched samples, cytosolic-enriched samples do not appear to separate by genotype on PC2. These data suggest drought impacts both organelle- and cytoplasm-enriched samples, and that the organelle drought response may be more genotype specific compared with cytosol profiles.

Novel genotype-specific responses may explain pre-flowering drought tolerance in RTx430

A comparison of significant drought-induced protein abundance changes in RTx430 with BTx642 identified multiple proteins that changed exclusively in RTx430 (Fig. 2 A, B). For example, the likely CDC73/PHP protein C5WQC9 was significantly >100-fold more abundant in RTx430 organelle-enriched samples in response to drought, with no significant fold change in BTx642. Similarly, a significant 65-fold drought-responsive increase was observed in the ADP-Glucose pyrophosphorylase (A0A1Z5R3X9) and a 34-fold increase in the likely Abscisic acid receptor PYL9 C5XZF6. Multiple drought-relevant proteins became significantly less abundant exclusively in organelle-enriched RTx430 during water deficit. A 45-fold decrease in the likely replication factor C protein A0A1Z5S8C0, 43-fold reduction in the likely phospholipase A2

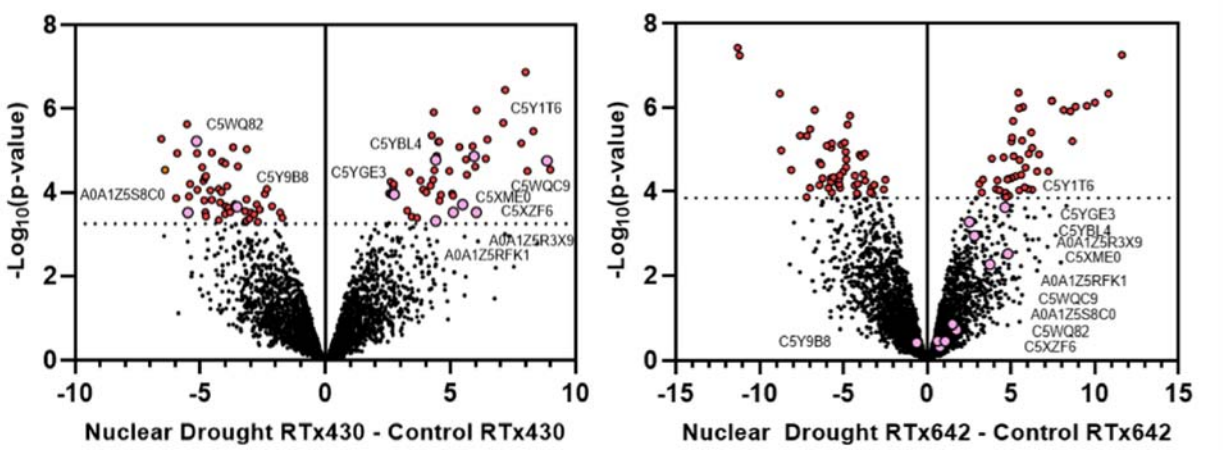

Figure 3. Comparison of genotype-specific drought responses in organelle-enriched samples of RTx430 (A) and BTx642 (B). Pink circles indicate proteins that changed significantly only in RTx430 (limma adj. p-value <0.05).

protein C5WQ82 (PLA2) and a 93-fold drought-induced decrease in the HMA domain-containing protein C5XTE0 were exclusively observed in RTx430 organelle-enriched samples.

Drought is the primary stress causing crop yield loss. Comparing closely related genotypes with differing drought tolerance has proven a successful way to identify plant adaptation strategies to water-deficit stress. Two Sorghum bicolor genotypes, RTx430 and BTx642 are characterized as pre-flowering drought-tolerant and -sensitive, respectively. Multiple reports have examined the differences between each genotype in response to water limitation but have relied on whole-organ and transcript-level measurements. To further understand RTx430's unique drought tolerance, we report here a close examination of the protein-level drought response in organelle- and cytosolic-enriched cellular compartments. A manuscript draft describing the above findings are currently in preparation.

Impact

To our knowledge, this study constitutes the first comprehensive protein profiling of Sorghum subcellular compartments in response to drought. Our findings suggest that genotype-specific changes in protein profiles may be greater in organelles, compared with the cytoplasm. The proteins identified as changing only in RTx430 during drought implicate multiple processes in RTx430's unique pre-flowering drought tolerance.

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