The Integration of Time of Day and Transcript Abundance in Sorghum in Chilling Stress

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Identifying transcripts that change in response to stress can provide important insights into how a plant perceives and responds to stress. Therefore, it is a widely-used practice to perform RNA-Seq as a first step to compare treatment and control plants and identify differentially expressed genes. However, the expression levels of transcription factors are regulated by the circadian clock. The time of day an experiment is done can have significant effects on the differentially expressed genes identified. The same response monitored at two times of day can show very different results, and it may give a more accurate picture to capture the response at multiple time points. However, transcription itself may be under the control of the circadian clock. If global transcription is altered either by time of day or the stress that the plant is exposed to, the standard approach of normalizing the total reads or counts per million can lead to the wrong interpretation of the data. A spike-in control is an external RNA with known sequence and quantity for calibrating RNA-Seq data. Adding spike-in controls can eliminate the bias from global transcriptional changes and allow accurate data interpretation. Here we evaluated how the time of day the plant response was examined and the RNA normalization approach affected the observed response of sorghum to early-season chilling stress. We found that using external RNA spikes as a normalization factor helped remove within-sample variations but captured variations between samples from different times of the day and chilling stress treatment. The differential expression analysis indicated an increase in the number of differentially expressed genes in the dataset normalized by external spikes. Our results suggested that normalizing RNA-Seq data with external RNA spikes provided a better insight into RNA-Seq data interpretation.