

# In-context Promoter Bashing of the *Sorghum bicolor* Gene Models Functionally Annotated as Bundle Sheath Cell Preferred Expressing Phosphoenolpyruvate Carboxykinase and Alanine Aminotransferase

## Background/Objective

Historically, several genetic approaches have been deployed in plants systems to modulate transcription of endogenous genes. In-context promoter bashing via genome editing is a route to identify and characterize critical regulatory regions that govern expression of genes of interest. This work describes the design and introduction of genome editing reagents into sorghum to create deletion of segments of DNA within the regulatory regions that control the expression of two genes involved in photosynthesis. The impact on the sorghum plant is presented.

## Approach

A set of *Streptococcus pyogenes* endonuclease (*SpCas9*)-based editing reagents was assembled and introduced into grain sorghum (RTx430) that carry six multiplex guide RNAs (gRNAs) designed to in-context promoter bash the 2 kb upstream of the translational start of gene models encoding for *SbPEPCK.BS* and *SbAlaAT.BS*. Selected edits of varying sizes in each of the respective upstream regions were characterized at the molecular level and phenotyped under greenhouse and field environments.

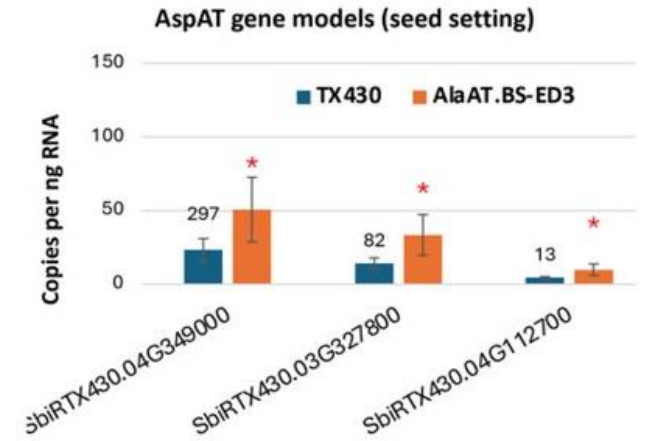
## Results

A 138 bp within the *SbPEPCK.BS* upstream region and a 1643 bp element within the *SbAlaAT.BS* upstream region were determined to be important for maintenance of transcription levels. No change in development or various physiological parameters was observed in characterized lineages carrying promoter edits. However, significant changes in seed reserves and a reduction in 100-seed weight were consistently observed in plants carrying an edit in the promoter of *SbPEPCK.BS* gene, which were significantly reduced in transcript accumulation for this gene.

## Significance/Impacts

The outcomes of in-context promoter bashing can be used to inform editing strategies to modulate the expression of selected gene models in a desired fashion. The six-plex guide design implemented is robust, with dual edits observed at a workable frequency. Indels within regulatory regions in sorghum can be created that impact expression and phenotypic outcomes. The bundle sheath enriched *SbPEPCK* (*sorghum bicolor* phosphoenolpyruvate carboxykinase) gene model, carrying an edit that results in significant downregulation, impacts total protein in kernel reserves and seed size.

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**Monitoring of transcript levels across selected *SbAspAT* gene models in the edited lines *SbPEPCK.BS-ED2* and *SbAlaAT.BS-ED3* at seed setting stage.**