

# Comparing Four Heat-inducible Promoters in Stably Transformed Sugarcane Regarding Spatial and Temporal Control of Transgene Expression Reveals Candidates to Drive Stem-preferred Transgene Expression

### **Background/Objective**

Inducible promoters provide remarkable utility when sustained transgene expression compromises plant development or agronomic performance. In this study, four different plant heat shock protein promoters were characterized in the vegetative tissues of stably transformed sugarcane to evaluate their efficacy and spatial expression profiles when directing the expression of a *uidA* reporter gene.

# **Approach**

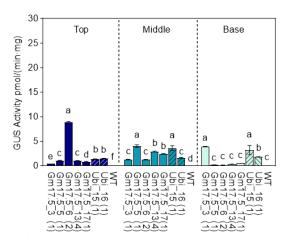
To evaluate their utility, GUS reporter gene expression driven by four plant-sourced HSP promoters (pGmHSP17.5, pHvHSP17, pZmHSP17.7, and pZmHSP26) was compared across various tissues of stably transformed sugarcane before and after heat treatment.

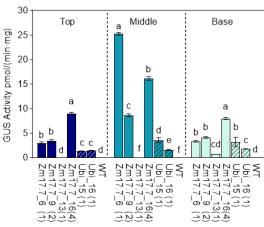
### **Results**

Heat-induced reporter gene activity in stem mid-sections of single-copy transgenic lines containing pZmHSP17.7, pHvHSP17, or pZmHSP26 exceeded pZmUbi-derived *uidA* activity by 9.7-fold, 3.8-fold, and 3.0-fold, respectively, with 346- to 3,672-fold induction compared to control conditions. Most promoters showed peak expression in middle sections of the stem, while pHvHSP17 was most active in stem apices. Histochemical analysis revealed that pZmHSP17.7 and pHvHSP17 were active in both parenchyma cells and vascular bundles within sugarcane stems.

# Significance/Impacts

This study produced new quantitative knowledge on the temporal and spatial expression of HSP promoters in sugarcane, thus expanding the promoter toolbox for crop biotechnology. These findings will support gene function studies and biotechnology applications, including heat stress tolerance, controlled transgene expression, precision gene editing, and complex metabolic engineering.





GUS activities in HSP lines and WT after heat treatment compared with that in Ubi lines in different stem sections (top, middle and base of stem). (a) pGmHSP17.5 lines. (b) pZmHSP17.7 lines. pHvHSP17 and pZmHSP26 not shown. uidA copy numbers are shown in parentheses following the line IDs. Different letters indicate significant difference at p < 0.05 according to Fisher's least significant difference (LSD) comparison.

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