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CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Synthetic Feed-forward Loop Circuit Boosts Transgene Expression in Sugarcane

Background/Objective

Bio-based products derived from natural plant-derived materials offer a promising alternative to petroleum-based products, which are essential for achieving global sustainability. Metabolic engineering of sugarcane for hyperaccumulation of biomass oil is emerging as a strategy to elevate the crop's energy content. Synthetic transcription factors offer a powerful tool for modulating entire metabolic pathways by enabling fine-tuned activation or repression of specific genes. However, the application of these technologies faces challenges, including the availability of well-characterized genetic building blocks for precise manipulation of gene expression, and the inherently challenging properties of gene expression in highly polyploid crops like sugarcane. In this study, we designed and built a coherent feed-forward loop circuit for plants and evaluated its performance in sugarcane calli, leaves, and stems.

Approach

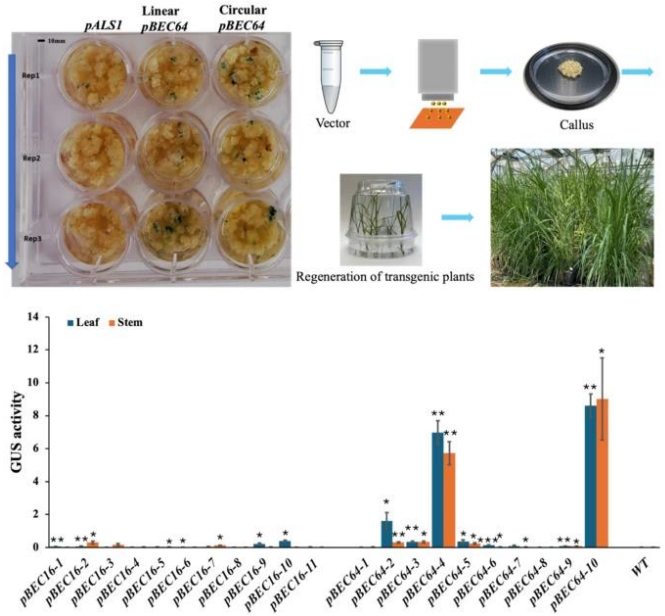
A version of the GAL4 system, originally developed for enhancer trap assays in Arabidopsis (Engineer et al. 2005), and the EDLL transcription activation domain (Tiwari et al. 2012) were combined with the promoter of the *Dry* locus in sorghum (Fujimoto et al. 2018), *pSbDry*, to control the expression of the GUS reporter gene as a coherent feed-forward loop circuit (*pBEC64)*. *pBEC64* and *pBEC16* were compared in stably transformed sugarcane plants.

Results

Among the transgenic events, 45.5% (5/11) and 27.3% (3/11) of *pBEC16*, and 70.0% (7/10) and 80.0% (8/10) of *pBEC64* showed significantly higher GUS activities than the wild type (CK) in leaf and stem tissues, respectively. These findings provide evidence that the GAL4-BD:EDLL artificial transcription factor is functional, and that the feed-forward loop construct (*pBEC64*) is effective at enhancing gene expression in sugarcane.

Significance/Impacts

Elevating transgene expression in sugarcane has great potential for pathway engineering to fuel the emerging bioeconomy. Future applications could involve adapting this circuit to regulate different target genes involved in metabolic engineering, stress resilience, or developmental pathways, and expanding its use to other crops by customizing the promoter or transcriptional activator components to match species-specific expression profiles.



Evaluation of the synthetic feed-forward loop circuit in sugarcane.

Zhang et al. 2025. "Synthetic Feed-forward Loop Circuit Boosts Transgene Expression in Sugarcane." *Plant Cell Reports*. DOI: 10.1007/s00299-025-03561-3.