Building a strong Cas9 transcriptional activator for plants

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A library approach to building a strong plant activation domain

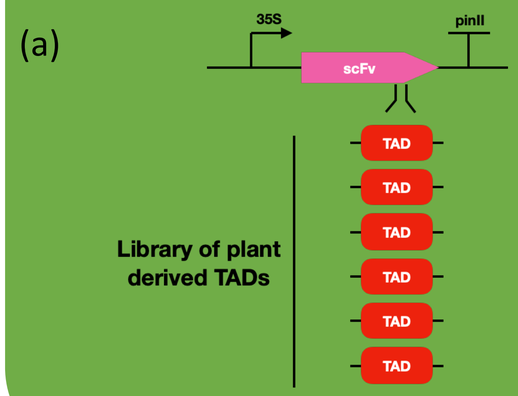
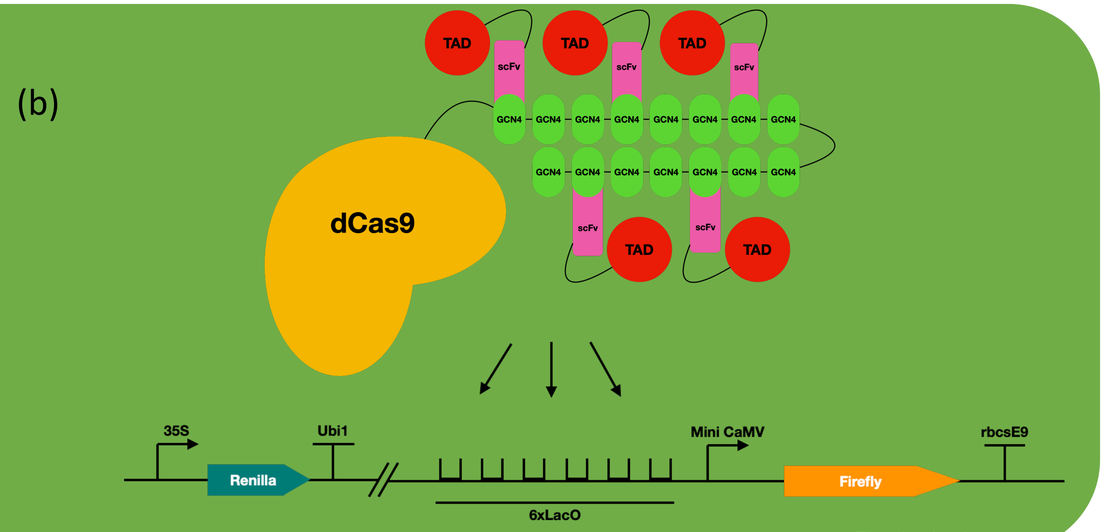


Figure 1: Design, Build, Test Strategy Towards a Strong Plant Activator. A library of putative plant-derived transactivation domains (TADs) was synthesized, and DNA fragments were cloned via Golden Gate assembly into the SunTag CRISPR-dCas9 transcription activation system[1,2] (1a). Protoplast cells were then isolated from *Setaria viridis* and transformed with plasmids encoding the Cas9-SunTag system, along with a dual luciferase plasmid containing an activatable minimal promoter to quantify TAD strength (1b).



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DREB1, DREB2, and HSFA6b drive strong activation of a luciferase reporter in *Setaria viridis* protoplasts

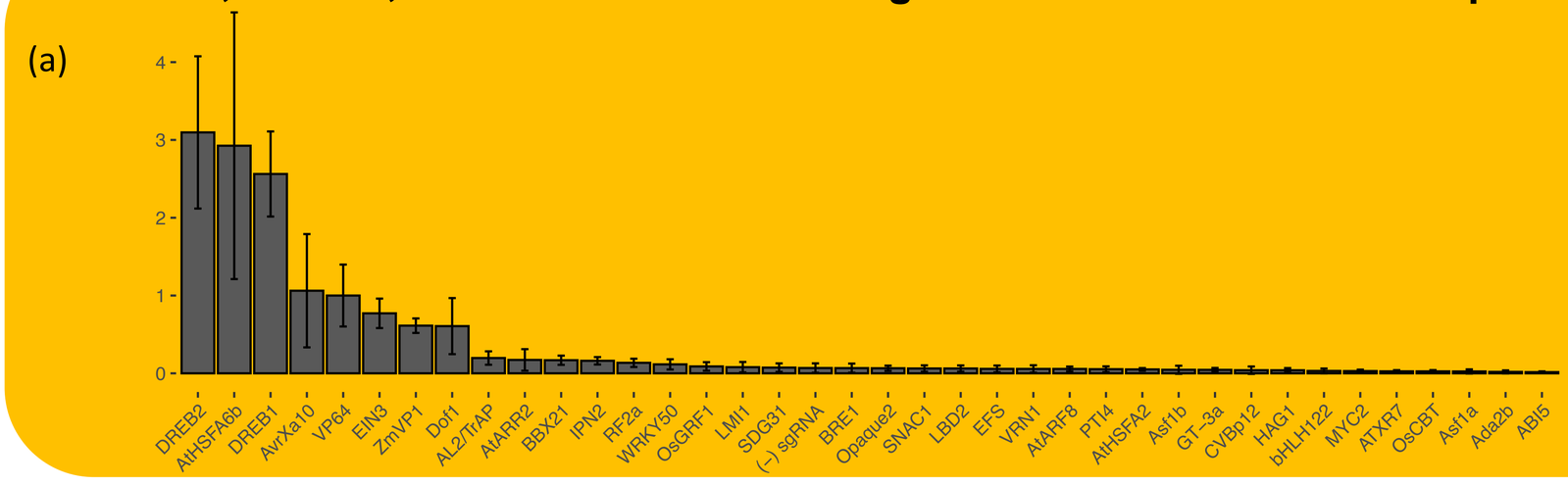

Figure 2: Three plant-derived stress response transcription factor TADs drive strong reporter activation: The library of cloned TADs were transformed into protoplasts and crude lysate was prepared 24h post transformation. Luminescence was measured and fold change was calculated relative to a negative control lacking a sgRNA targeting the minimal promoter. The TADs derived from DREB1, DREB2, and HSFA6b transcription factors drove strong activation (2a).

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DREB2 outperforms VP64 across three endogenous target genes in Arabidopsis thaliana protoplasts

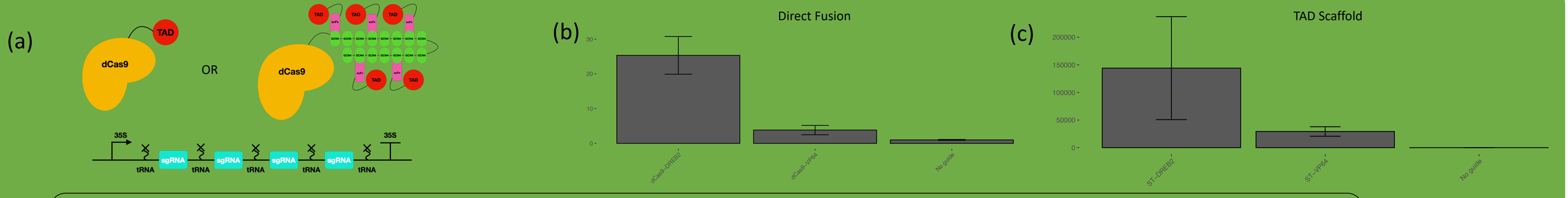

Figure 3: The DREB2 TAD drives stronger activation than VP64 in both direct fusion and TAD scaffolding architectures: Constructs containing dCas9 directly fused to VP64 and DREB2,SunTag-VP64 and SunTag-DREB2 were generated driven by a strong promoter. In addition, a multi-guide array was constructed for three endogenous target genes in Arabidopsis to test the ability of the different systems to activate transcription at endogenous loci (3a). The DREB2 domain outperforms VP64 as both a direct fusion (3b) and a TAD scaffold (3b).

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1. Tanenbaum, M. E., Gilbert, L. A., Qi, L. S., Weissman, J. S. & Vale, R. D. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. *Cell* **159**, 635–646 (2014).
2. Papikian, A., Liu, W., Gallego-Bartolomé, J. & Jacobsen, S. E. Site-specific manipulation of Arabidopsis loci using CRISPR-Cas9 SunTag systems. *Nat. Commun.* **10**, (2019).

Funding Sources