Interaction of Class IV Homeodomain Leucine-Zipper Transcription Factors and GIR Adapter Proteins in *Arabidopsis*

USDA National Institute of Food and Agriculture
U.S. DEPARTMENT OF AGRICULTURE

[1] Lillian Marberg, [2] Kathrin Schrick

[1]College of Saint Benedict, MN; [2]Division of Biology, Kansas State University, KS

Background

Homeodomain Leucine-Zipper Transcription Factors

- Regulate cell differentiation in *Arabidopsis*
- Steroidogenic Acute Regulatory related lipid transfer adjacent domain (**STAD**)
- STAD domain hypothesized to be important in protein binding

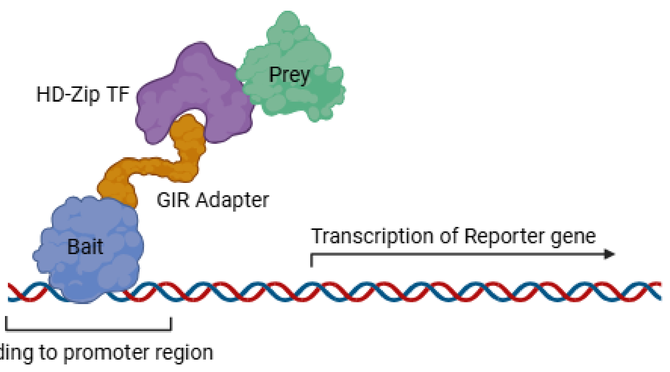
GIR1, GIR2, SIED 1 Adapter Proteins

- Produced as a stress response and affect root hair regulation and giant cell formation in the sepal
- Contain a cysteine motif predicted to form a zinc finger
- Zinc Finger hypothesized to be important in protein binding

Objectives

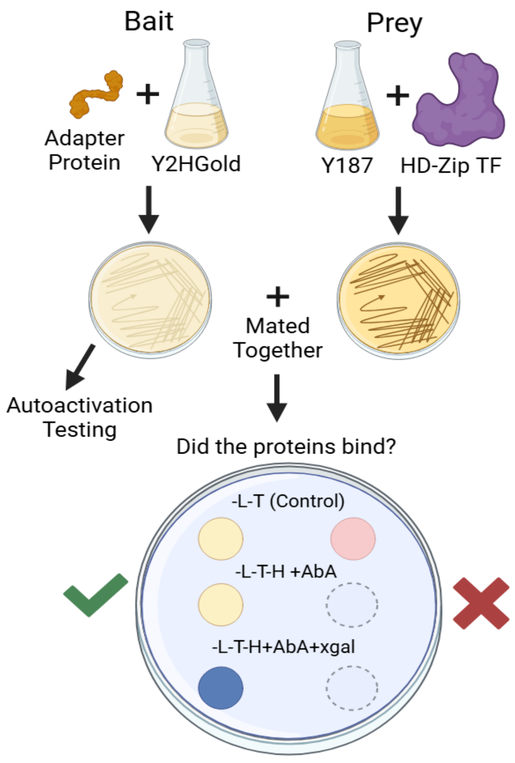
- **Determine all binding and non binding combinations between HD-Zip TFs and GIR adapter proteins using a Yeast 2 Hybrid assay**
- **Identify possible amino acid sequences responsible for discrepancies in binding.**

Experimental Design



- **(-L-T)** –Leucine -Tryptophan as control
- **(-L-T-H+AbA):** -Leucine –Tryptophan –Histone with Abscisic Acid

- If complex **is formed** we should see growth on all plates
- If complex is **not formed** we should see growth only on –L-T control plate.



3D Predicted Structures

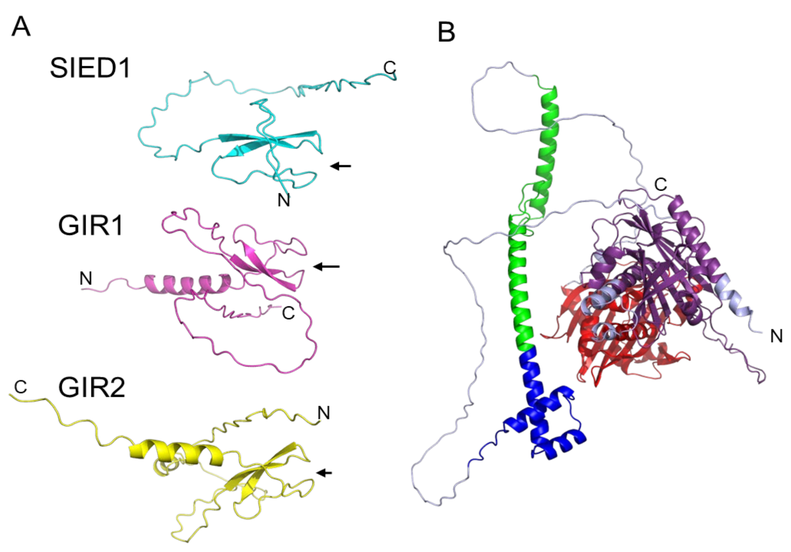
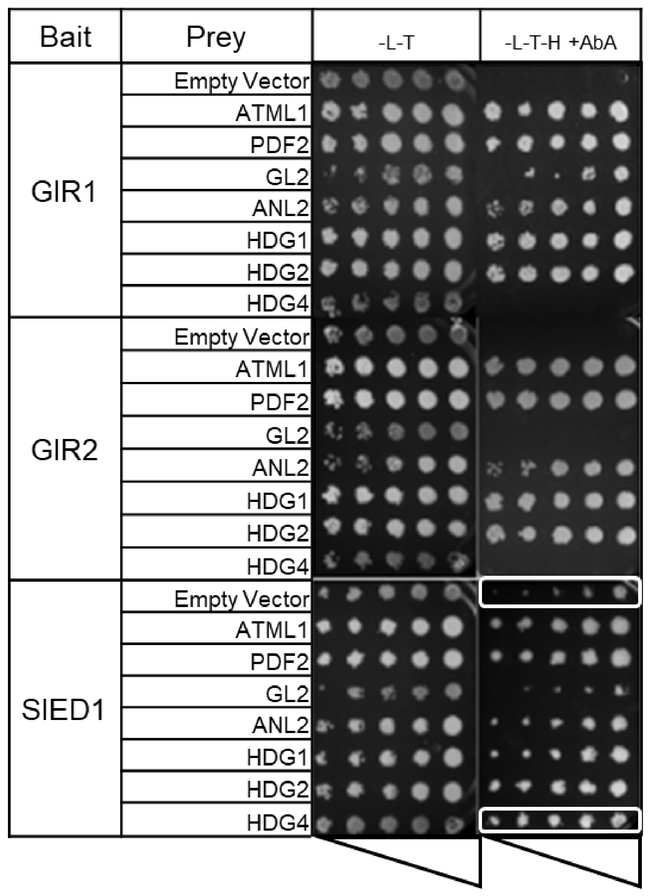


Figure 1: (A) SIED1, GIR1, GIR2, with Zinc Fingers denoted. **(B)** PDF2 Domains with STAD highlighted in purple.

Bait and Prey Frogging



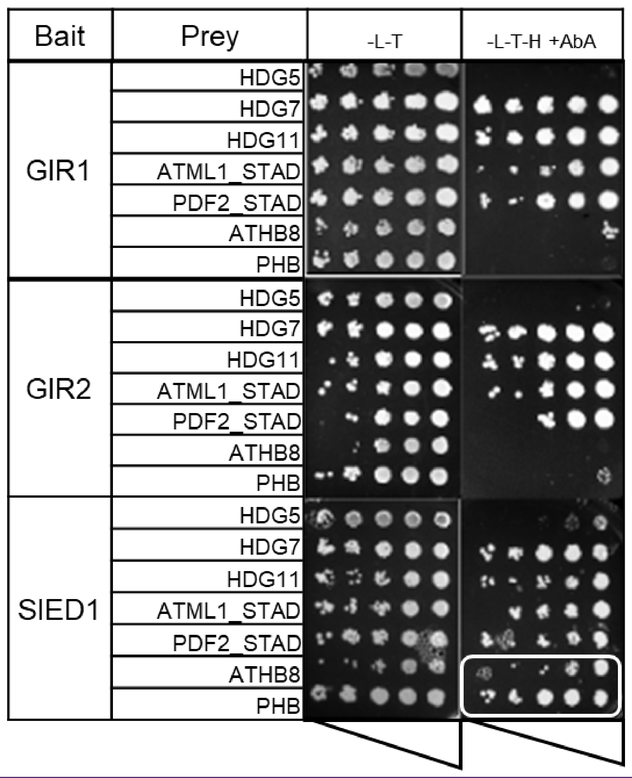


Figure 3: Frogging of all bait prey interactions on –L-T plates that confirm presence of plasmid, and –L-T-H+AbA that confirm if the proteins bound. SIED1 autoactivation could potentially explain discrepancies in binding. GIR1 and GIR2 have almost identical binding patterns.

Autoactivation Results

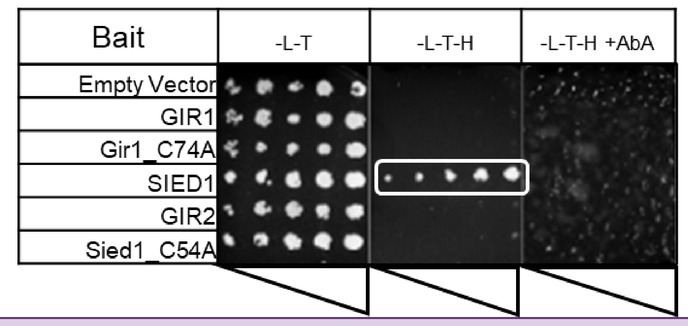


Figure 2: -L-T confirms presence of plasmids, -L-T-H and –L-T-H+AbA tests autoactivation with –L-T-H+AbA being more selective. Slight autoactivation shown by SIED1.

Interactions

		Bait		
		GIR1	GIR2	SIED1
Preys	Empty Vector			
	ATML1			
	PDF2			
	GL2			
	ANL2			
	HDG1			
	HDG2			
	HDG4			
	HDG5			
	HDG7			
	HDG11			
	ATML1_STAD			
	PDF2_STAD			
	ATHB8			
	PHB			

Figure 4: Table of bait-prey interactions. Deeper purple denotes stronger interaction, and no color means none.

Amino Acid Sequences

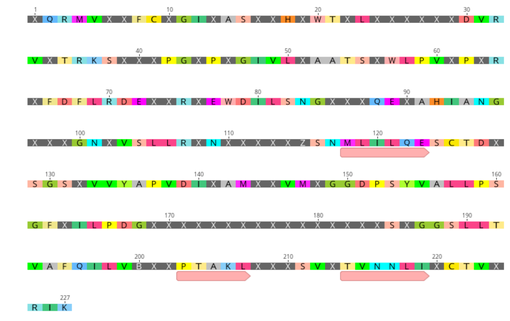


Figure 5: Amino Acid consensus of all HD-Zip TF STAD with 75% frequency being the cutoff. Motifs contained in only bonding TF's highlighted

Conclusions

- **Possible interaction with SIED 1 and Class III HD-Zip TF's**
- **GIR1 and GIR2 have identical binding patterns.**
- **Future Work: Substitution mutations in identified amino acid sequences to determine importance in binding**

Acknowledgements

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