IOWA STATE UNIVERSITY
Genetics, Development, and Cell Biology

Iowa State University Honors Program
Fall 2020
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EXA1 is required for Arabidopsis hypocotyl growth

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Rationale

Auxin is a key plant hormone that causes rapid changes in gene expression, but it is not understood how this occurs post-transcriptionally.

EXA1 is an Arabidopsis protein involved in translation that is regulated by auxin and required for auxin mediated shoot elongation.

Hypothesis: EXA1 is required to attenuate auxin responses in the shoot via the AFB4 and AFB5 auxin receptors but not TIR1.

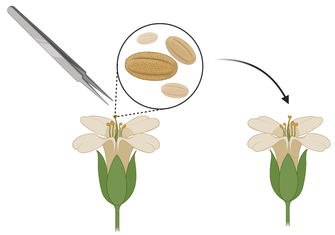
Objectives: Examine if EXA1 regulates the abundance of particular auxin receptors in shoots using genetics and microscopy.

Methods

- Generating double, triple and a quadruple mutants containing *exa1* and three auxin receptors *(tir1, afb4*, *afb5*)
- Crossed fluorescently tagged auxin receptors (TIR1-Venus, AFB4-Citrine and AFB5-Citrine) in *exa1* to examine auxin receptor levels in the *exa1* mutant
- Crossed the auxin reporter DR5:GFP in *exa1* to visualize auxin responses *in vivo*.
- Extracted DNA from >600 individuals from F1 to F2 populations to genotype each plant.
- Utilized polymerase chain reaction (PCR) and gel electrophoresis methods in order to genotype individuals.

Results

F2 populations in progress from initial crosses performed:



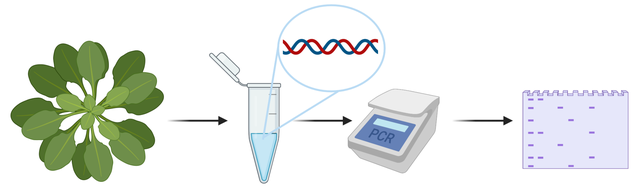
A) TIR1-VENUS x *exa 1-1*
B) AFB4-CITRINE x *exa 1-1*
C) AFB5-CITRINE x *exa 1-1*
D) DR5:GFP x *exa1-1*
E) tir1-1 afb4-8 afb5-5 x exa1-1

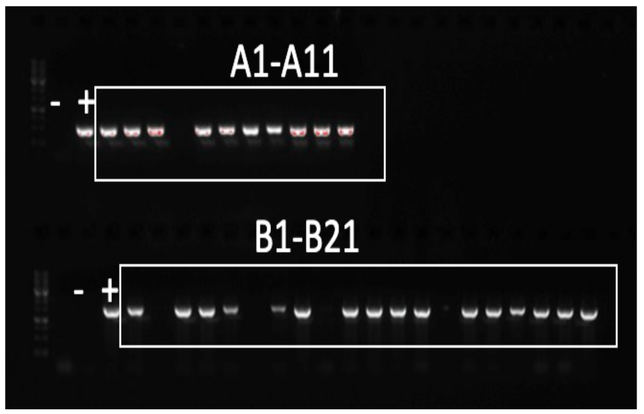
A) Example results for genotyping for TIR1-VENUS transgene in F2 plants:

- Negative control (-) is water, the positive control (+) is DNA extracted from TIR1-VENUS transgenic control line. 10/11 plants have the transgene and are homozygous for the *exa1-1* allele.

B) Results for genotyping for AFB4-CITRINE transgene in F2 plants:

- Negative (-) control is water, the positive control is DNA extracted from the AFB4-Citrine transgenic control line. 16/20 individuals carry the transgene and are homozygous for the *exa1-1* allele.





Conclusion and Outlook

I have genotyped >100 F1 plants and >500 F2 plants to identify genotypes of interest for phenotyping and microscopy. During Spring 2021 I will be able to plant these novel mutants and phenotype them in the presence and absence of auxin to test our hypothesis. I will also use confocal microscopy to look at auxin receptors and reporter accumulation in the absence of *EXA1*. With this information, we will be able to further our understanding of hormone regulation of gene expression in Arabidopsis.

References:: Hashimoto et. al., 2016 Plant Journal, Matsui et. al., 2017 PLoS Genetics; Wu et. al., 2017 eLife; Kelley et. al., 2017 bioRxiv. This project was supported by ISU start-up funds to D.R.K., a Fung Fund Genetics Summer Internship and an LAS Dean's High Impact Award to K.H. (Fall 2020). We wish to thank Sarah Loo and Sarah Smith for their assistance with this project. Cartoon figure created using BioRender.