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LOF1 and Interacting Transcription Factors in Plant Development and Crop Yield

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Abstract
Transcription factors (TFs) help ensure proper gene expression in developing tissues, and thus play a role in plant development and plant architecture. LATERAL ORGAN FUSION1, or LOF1, is a TF expressed in the organ boundaries of Arabidopsis thaliana. lof1 mutants have fused axillary branches and cauline leaves, which indicates importance in boundary development. Because transcription factors are known to act in complexes, we wanted to discover what other proteins interact with LOF1. We executed a yeast-2-hybrid (Y2H) screen that identified several TFs as potential interactors: WHIRLY 3 (WHY3), MYB DOMAIN PROTEIN32 (MYB32), HOMEOBOX-LEUCINE ZIPPER PROTEIN4 (HB4), and LIGHT RESPONSE BTB2 (LRB2). WHIRLY1 (WHY1) and HOMEOBOX ARABIDOPSIS THALIANA3 (HAT3) are thought to be redundant with WHY3 and HB4, respectively, and are included in our study. To gain evidence that the interactions between the potential protein interactors and LOF1 is biologically relevant in planta, we will characterize T-DNA insertion lines in which the genes that encode these interactors are disrupted. Our goal is three-fold: genotype the T-DNA lines to identify homozygous mutants; characterize the phenotypes of these mutants and compare to known phenotypes; and create double- and triple-mutants between lof1 and the other TFs. Because the boundary region is involved in determining leaf angle and leaf angle affects planting density, changes in leaf angle have the potential to impact crop yield. In the future, we may be able to apply the knowledge we obtain in the model plant Arabidopsis thaliana to crop species in order to improve crop yield.

Keywords
molecular biology, plant research, PCR, LOF1, transcription factors

Disciplines
Biology | Cell Biology | Plant Biology

Comments
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LOF1 and Interacting Transcription Factors in Plant Development and Crop Yield

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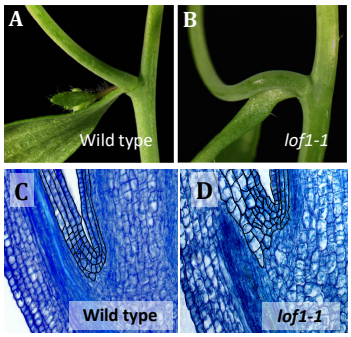
Introduction

Transcription factors (TFs) help ensure proper gene expression in developing tissues, playing a role in plant development and plant architecture. LATERAL ORGAN FUSION1, or LOF1, is a TF expressed in organ boundaries of *Arabidopsis thaliana*. Because TFs are known to act in complexes, we want to discover what other proteins interact with LOF1. A yeast-2-hybrid (Y2H) screen identified several potential protein interactors (listed below). To explore the role of these proteins in LOF1-related processes *in planta*, we obtained T-DNA insertion lines of the genes that encode these proteins. Because the boundary region is involved in determining leaf angle and leaf angle affects planting density, understanding how boundary proteins interact to affect leaf angle has the potential to improve crop yield.

lof1 mutant phenotype

lof1 mutants have fusion between the axillary branch and cauline leaf and abnormal boundary cell division and growth.



Lee and Springer 2009

Interacting Transcription Factors

• TFs identified in the Yeast-Two-Hybrid (blue). TFs that are thought to be genetically redundant with the Y2H identified TFs (black)

-WHIRLY 3 (WHY3) and WHIRLY 1 (WHY1)

-MYB DOMAIN PROTEIN 32 (MYB32)

-HOMEOBOX-LEUCINE ZIPPER PROTEIN 4 (HB4) and HOMEOBOX ARABIDOPSIS THALIANA3 (HAT3)

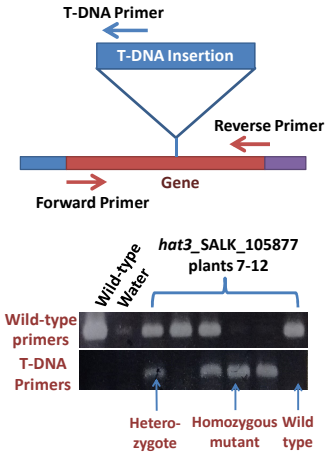
-LIGHT RESPONSE BTB 2 (LRB2)

Genotyping T-DNA Insertion Lines

Plants were genotyped using Polymerase Chain Reaction (PCR) to identify homozygous mutants.

1. Gene-specific primers amplify wild-type product if wild-type allele is present; T-DNA primer and one gene-specific primer amplify T-DNA product if insertion allele is present.

2. Comparison of amplified PCR products from both reactions indicates the genotype of the plants tested (example).

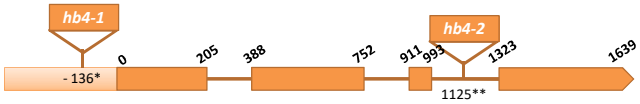


T-DNA Insertion Site Identification

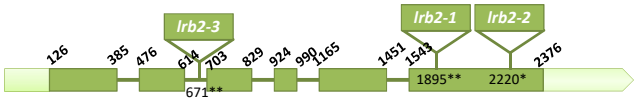
•Insertions sites were confirmed via sequencing of PCR products containing genomic DNA flanking left border.

•HOMEOBOX ARABIDOPSIS THALIANA 3 (HAT3)

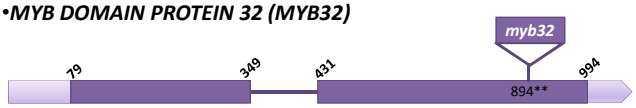
•HOMEOBOX-LEUCINE ZIPPER PROTEIN 4 (HB4)



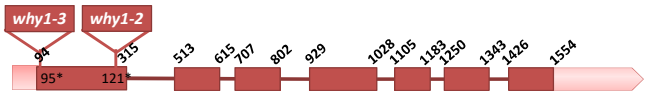
•LIGHT RESPONSE BTB2 (LRB2)



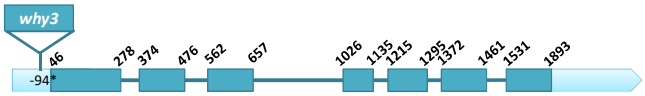
•MYB DOMAIN PROTEIN 32 (MYB32)



•WHIRLY 1 (WHY1)



•WHIRLY 3 (WHY3)



*Insertion sites were sequenced.
**Insertion sites were identified through TAIR.
Figures are not drawn to scale. Numbers correspond to bp, beginning from transcription start site.

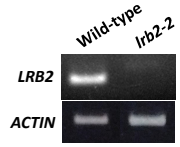
Figure Legend:

= Exon
= Intron
= 5' UTR
= 3' UTR
= T-DNA Insertion Site

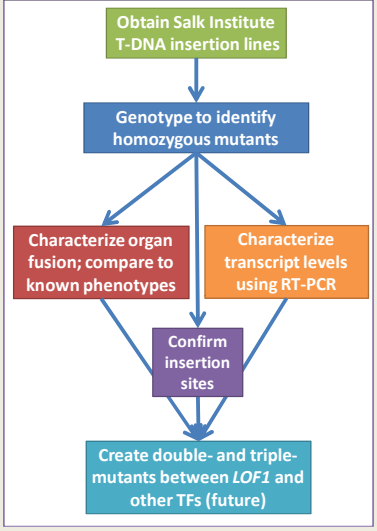
Transcript levels in homozygous mutants

Semi-quantitative RT-PCR was used to determined the level of transcript in homozygous mutants.

Intensity of band correlates to the level of mRNA transcript accumulated. Lack of a band from *lrb2-2* suggests that it is likely a null allele.



Experimental Design



Future Directions

•Create double and triple mutants between *lof1* and the homozygous T-DNA mutants identified in this study

•Resulting phenotypes will give support for LOF1-interacting TFs' involvement in LOF1 processes

•Gain a better understanding of how *LOF1* functions

•May be able to apply this knowledge to crop species to improve crop yield

Acknowledgements

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References: Lee, D. K., M. Geisler and P. S. Springer (2009). Development **136**(14): 2423-2432.

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