



Downstream Effects of Alternative Splicing of HRE1 in *Arabidopsis thaliana*



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Introduction

- HRE1 is upregulated during anoxic (flooded) conditions but its downstream effects on other metabolic pathways have not yet been investigated.
- As the hydrology of Florida changes, HRE1 is likely to be implicated in plant's adaptation to new conditions.
- The current rate of sea level rise is 3.2 mm per year, but this value continues to increase¹. As sea level rises, effects of saltwater intrusion on native ecosystems and agriculture move inland. Extreme weather events such as severe hurricanes and high tide flooding have increased 400% since 2004¹.

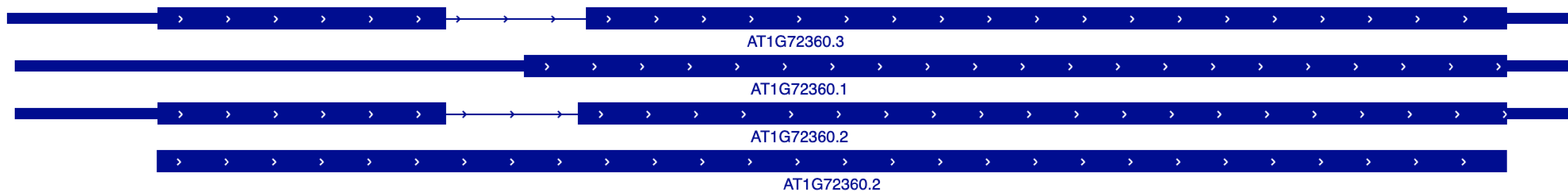
Hypothesis

Does alternative splicing of AtERF73/HRE1 affect immune response metabolism in *Arabidopsis thaliana*?

Background

- HRE1 α and HRE1 β are 2 alternative splicing variants of HRE1.
- HRE1 is a transcription factor in the 'Apetala 2' and 'Ethylene Response Factor' (AP2/ERF) superfamily. It can bind as either a repressor or an activator².
- Mutants with overexpression of HRE1 have recently been shown to have increased resistance to anoxic conditions through upregulation of low oxygen signaling, anaerobic and ethanol fermentation pathways².
- HRE1 β is strongly expressed during hypoxic conditions while HRE1 α is expressed during low stress conditions.

• **Figure 1:** Visualization of HRE1 α (top) and HRE1 β (2nd from top) exon and intron organization. (HRE1 γ and HRE1 δ pictured for reference as well).



Methods

- Public, single end, RNA sequencing data was obtained from the National Center for Biotechnology Information (NCBI).
- Quality assurances including filtering and trimming were performed using the program, SAMstat.
- Reads were aligned and mapped to the *Arabidopsis* genome using programs Bowtie2 and Tophat.
- Quantification was performed using the pseudo aligner, Kallisto.
- Differential Gene expression analysis was completed in Rstudio using DESeq and EdgeR, EDASeq, and NOISeq packages.

Data

Gene	Pathway	Log Fold Change
ICS1/SID2	Salicylic Acid	-6.11
PAD4	Salicylic Acid	-3.51
DND1	Hypersensitive Response	-2.98
PEN3/PDR8	Syntaxin/exocytosis	-2.12
PEN2	Hypersensitive Response	-1.07

Differential Expression Overview

Number of Differentially Expressed Genes	
Upregulated ($\beta > \alpha$)	132
Downregulated ($\beta < \alpha$)	551

Results

- Expression of non-specific immune related genes was generally depressed in HRE1 β as compared to HRE1 α .
- Of all immune pathways, the salicylic acid pathway was most significantly affected (downregulated in plants with isoform HRE1 β as compared to HRE1 α).
- HRE1 β caused strong down regulation of immune mechanisms stemming from transportation/exocytosis of cytotoxins.

Discussion

- Hypoxic conditions, which most frequently result in expression of HRE1 β over HRE1 α may also result in both decreased specific (HR related) non-specific (SA related) immunity.
- Increased disease prevention and control mechanisms may be necessary if flooded conditions should arise in production agriculture environments.
- More research is needed to determine how sea level rise, extreme weather and tides will affect coastal agriculture.

References

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2. Gutterson N, Reuber TL. Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol. 2004 Aug;7(4):465-71. doi: 10.1016/j.pbi.2004.04.007. PMID: 15231271.
3. Alternative Splicing in Plant Genes: A Means of Regulating the Environmental Fitness of Plants, DO - 10.3390/ijms18020432 International Journal of Molecular Sciences