Reverse Genetics: Using phenotypic data from *Arabidopsis Thaliana* TDNA knockouts to make inferences about gene function

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Introduction

Although, *Arabidopsis thaliana* was the first plant to have its genome fully sequenced and is revered as a model in molecular biology and genetics[1], little is known about how and what genes cause the phenotypes observed both in the wild and a laboratory environment. As a genetic model, *A .thaliana* is an ideal plant to study the genetic mechanisms of salt tolerance by randomly knocking out expression genes and examining the resulting phenotypes – a process known as reverse genetics[2]. As saline soil environments tend to result in lower crop yields, understanding the genetic mechanism of salt tolerance could lead to the development of genetic modifications that would help plants grow in some of the harshest environments on Earth.

These experiments are part of a larger project: **unPAK** (undergraduates phenotyping of *Arabidopsis* knockouts) that aims to determine the roles of genes in producing phenotypes in a variety of ecological conditions through undergraduate driven research[3].

Research Objective: The goal of our two experiments was to determine whether the missing genes in *A. thaliana* knockouts, when compared to the controls or non genetically altered genotypes, contributed to an observable difference in the phenotypes produced while plants experienced different sources and varying concentrations of salinity stress.

- A growth chamber experiment focussed on whether the knockouts phenotypes, when compared to the controls, would show significantly that they could not function with increases in concentration of the same type of salinity.
- A greenhouse experiment asked whether the source of salinity would affect the plants tolerance and used the same concentration of different types of salinity.

Methods

Salinity Concentration Experiment growth chamber	Salinity Type Experiment Greenhouse
16hr light/ 8hr dark cycle, 22° C	Ambient
Grown in soil	Grown in 50/50 sand soil mix
3 replicates of 40 knockouts lines across two chambers	6 replicates of 20 (out of the 40) knockout lines
40mm NaCl, 80 mm NaCl, distilled water control	40 mm NaCl, Instant Ocean, Sea water, distilled water control

Fig 1. All seeds experienced a one week cold treatment before being sowed directly onto the soil

Fig 2. *Arabidopsis* germinant 2-3 days old. Ready for treatment acclimation

Fig 3. Plants have just started bolting a sign they are entering the reproductive phase.

Fig 4. Trays were rotated regularly to account for positional effects.

Fig 5. Bolt was recorded once bolt height was equal or greater than 5 mm.

Fig 6. Flowering phenology was recorded for all greenhouse plants.

Fig 7. Treatment continued until each plant senesced or died. The plant shown here has reproduced but died prematurely.

References. 1-3 Goals of the unPAK project. (2015). Retrieved August 13, 2015, from http://arabidopsisunpak.org/?page_id=46
4- All literature function for the SALK lines was found here: Alonso, José M, Anna N Stepanova, Thomas J Leisse.et al. "T-DNA Express: Arabidopsis Gene Mapping Tool." *SIGnAL Salk Institute Genomic Analysis Laboratory*. National Science Foundation, 8 Mar. 2015. Web. 13 Aug. 2015. http://signal.salk.edu/cgi-bin/tdnaexpress. 5: Chin, K., DeFalco, T., Moeder, W., & Yoshioka, K. (2013). The Arabidopsis Cyclic Nucleotide-Gated Ion Channels AtCNGC2 and AtCNGC4 Work in the Same Signaling Pathway to Regulate Pathogen Defense and Floral Transition1. *Plant Physiology, 163*(2), 611-624. 6-7:Xiang, Chengbin, Bonnie L. Werner, E'Lise M. Christensen, and David J. Oliver. "The Biological Functions of Glutathione Revisited in Arabidopsis Transgenic Plants with Altered Glutathione Levels." *Plant Physiology*126.2 (2001): 564-74.

Results and Discussion

The salinity concentration experiment is still in progress. Data presented here is for the salinity-type, greenhouse experiment only.

Germination	Days to Bolt	Rosette Diameter	Total Branches	Aborted fruit
87% June 4th 2015 ± 2 days	Mean=22.1 +/- 2.99 days	Mean= 39.7mm	One to eight	All plants had aborted fruit

Table 1. Difference between treatments within mutant genotypes was determined significant if the p-value (reported below for all significant tests; plus those that were marginally non-significant) was less than 0.05

SALK Accession	Trait	P-value	Function[4]
023690C	Rosette diameter Total branches	0.020 0.014	unknown function
026089C	Total branches Log_{10}(# fruit aborts)	0.018 0.001	Plasma membrane component, glutathione production
026568C	Days to bolting	0.007	Cyclic nucleotide-gated channel
026842C	Total # good fruit	0.042	Hypothetical protein
027037C	Height	0.029	unknown function
080708C	Log_{10}(# fruit aborts)	0.043	Glutathione transferase
106466C	Height Log_{10}(# fruit aborts)	0.039 0.023	oriented cell expansion in Arabidopsis

Note: Two knockout genotypes (three traits) with p-values >0.05, but less than 0.06 are not included in the table due to marginal non-significance but may be worth further investigation.

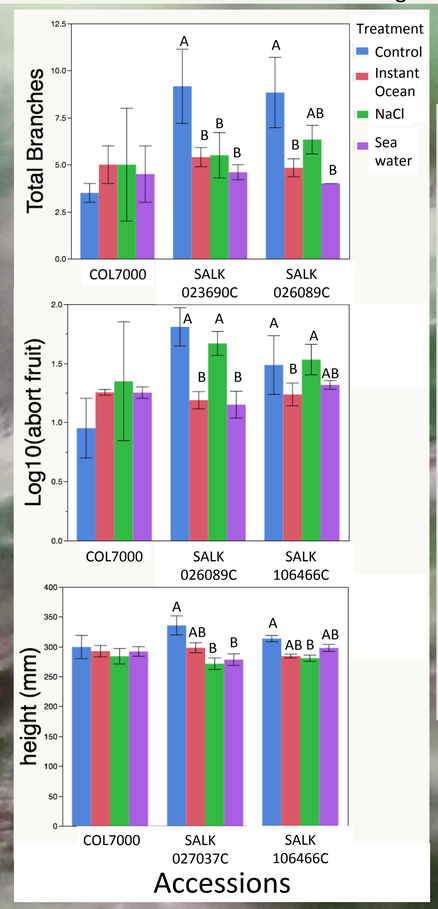

Fig 8. Phenotypic variation in (a) total branches, (b) failed fruit, and (c) height among treatments for one naturally occurring genotype (COL 7000) and two mutant lines per trait. It was expected that development would be more negatively affected when plants were stressed (with salt treatment or with normal stress response pathways mutated). It is interesting that salinity appeared to reduce growth (reduced height and branches) and fitness (more aborts), but phenotypes were not consistent across salinity treatments.

Note. Bars not sharing the same letter are sig different by Tukey's HSD tests performed within mutant lines per trait

The literature function of the genes knocked out in each mutant genotype may lead to inferences as to what caused the phenotypes observed. For example SALK_ 026568C, which was missing a gene related to cyclic nucleotide channels, showed significant variation for days to bolting where Instant Ocean treated plants bolted later than seawater and control water treated plants, but NaCl treated plants did not vary from any other treatment. As days to bolting is a major developmental stage in *A. thaliana,* and these channels have been implicated in Ca^{2+} signaling which is involved in development[5], the lack of this gene may explain why the treatments had the effect they did.

Conclusion

The experiment has shown that phenotypes may vary among sources of salinity, and that the loss of genes not known to be associated with salinity in *Arabidopsis* may explain some of the variation observed. Some of these genes are involved in antioxidant production and play a role in stress response, which is interesting and may explain why these plants had reduced growth and fitness under some salinity treatments. The knowledge gained from these experiments will be added to the unPAK data base, and will be used to help discover how genes affect phenotypic variation in the wild. The knowledge from these experiments can be applied to discovering the function of these genes and how they relate to salinity tolerance in plants, which could be applied to agricultural research.

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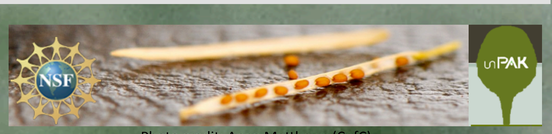

Photo credit: Anna Matthews (CofC)