# Effects of mutation on lateral root development in *Arabidopsis thaliana*


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## Introduction

- T-DNA insertions on *Arabidopsis thaliana* are performed using agrobacterium with the intent to cause loss-of-function mutations in a single gene region in order to investigate gene function (O'Malley and Ecker 2010).
- Mutant lines are listed for single insertions found by the SALK institute. However, not all of these insertions are unimutant. In fact, about 50% are not (Valentine et al. 2012).
- In this experiment, the root systems of unimutant, multi-gene, and natural accession were compared both on agar and across three phosphorus environments.
- Phosphate is an immobile soil nutrient that promotes lateral root development at the expense of primary root development (Williamson et al. 2001).

## Questions

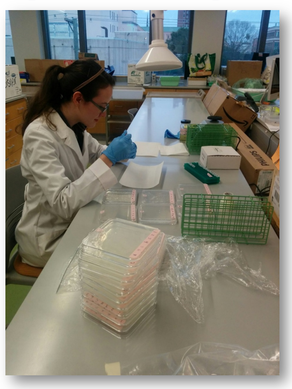
- Do insertions in or across multiple gene regions have more deleterious effects on plant phenotype?
- What are the effects of these gene regions on lateral root development?

## Agar Experiment: Methods

### Line Selection

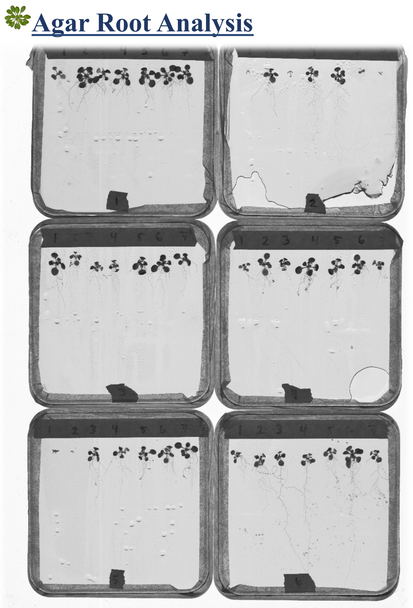
- Six lines with insertions in or across multiple gene regions
- Five lines from a previous root architecture study selected for greater root length
- Five randomly selected mutant lines from the same study
- Five natural accessions
- COL70000 as parental and scalar

### Experimental Design

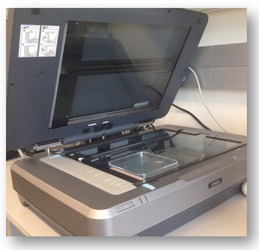


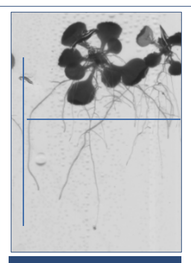
- Petri dishes filled with an agar medium
- Seeds placed according to randomized design
- COL70000 in the middle of each tray to act as a scalar
- Trays were cold treated for one week.
- Trays were allowed to grow for two weeks in a growth chamber.

### Agar Root Analysis



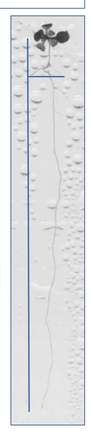
- Roots were scanned using an Epson scanner and WinRHIZO.
- Plants were judged on a scale of 1-5 units for primary root size and 1-3 units for lateral root size.





**SALK_057963C**

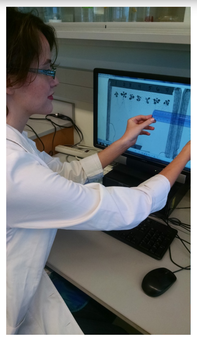
- Multi-gene line
- Primary root rating of 2
- Lateral root rating of 3



Scale:
Primary Root Unit
Lateral Root Unit

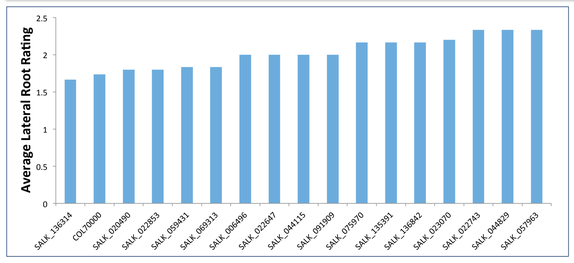
**COL 70000**

- Primary root rating of 5
- Lateral root rating of 1



### Mutant lines demonstrated greater lateral root ratings than COL70000


- Mutant lines show increased **and** decreased root performance in comparison to COL70000.
- Multi-gene influence lines (circled in blue) showed no trend separating them from other mutants lines in primary or lateral root growth.
- Lines representing four categories, table below, were chosen for further examination.



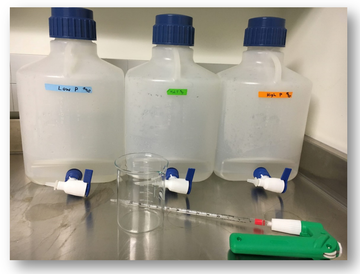

Rank order in lateral root production at 14 days.

| | Large Primary Root | Small Primary Root |
|---|---|---|
| Large Lateral Root | SALK_136842C<br>SALK_069313C | SALK_020490C<br>SALK_091909C |
| Small Lateral Root | SALK_059431C<br>SALK_075970C | SALK_006496C<br>SALK_044115C |

## Sand Experiment: Methods

### Design

- Phosphorous is known to have impacts on lateral root development
- Variations in available phosphorous could highlight phenotypes.
- Eight selected lines and natural controls were grown in three nutrient environments
- Harvested at 21 days of growth

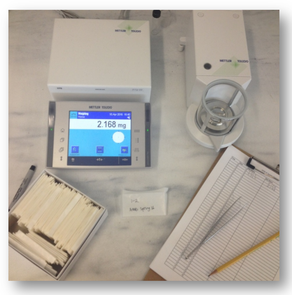


Nutrient solution pipetted onto sand

Randomized design

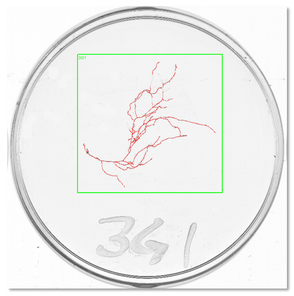
### Mass Analysis

- Aboveground
- Belowground
- Biomass allocation



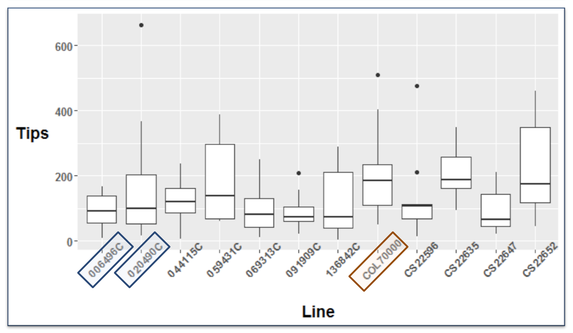
### Root Analysis

- Roots scanned using an Epson scanner and analyzed with WinRHIZO



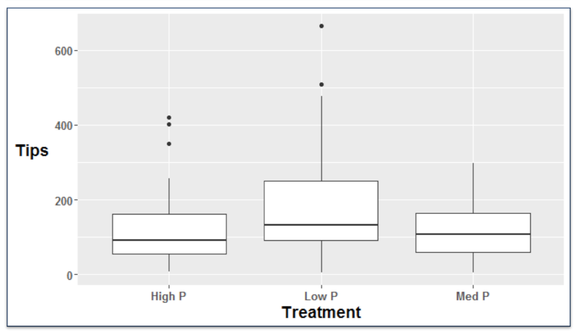
## Results

### Mutant lines had fewer lateral tips than COL70000.



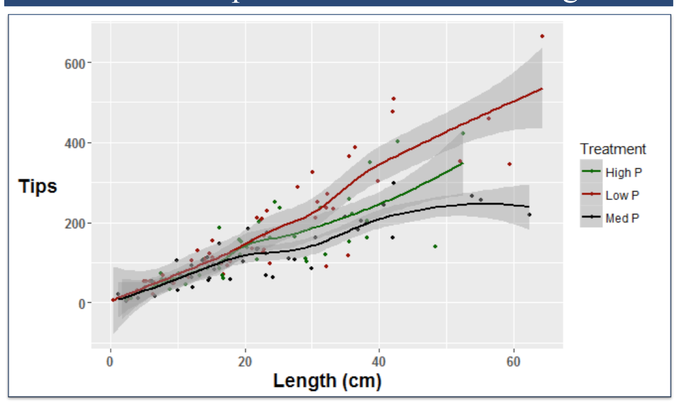

- Number of tips varies by line, ($F=2.24$; $P<0.01$).
- COL7000 had the greatest tip number.

### Low phosphorous treatments produced more tips.




- Number of tips varied significantly by treatment, ($F=5.19$, $p<0.007$)

### Number of tips increased with root length.




- Positive correlation between root length and tips, ($R^2=0.6945$).

## Going Forward

- Repeat of agar experiment with fewer lines per tray to in order to do a complete WinRHIZO analysis.
- Further subdivide lines into functional categories.
- Explore potential plant position and competition effects on agar and sand.

## Summary

- The Arabidopsis mutants used in this study showed greater lateral root ratings on agar, but fewer tips than natural accessions and control lines on sand.
- Multi-gene lines, noted on TDNA express to influence more than one gene, did not show a clear trend in primary or lateral root rating, and were roughly equivalent to other mutants in number of tips.
- Decreased phosphorous content of the growing medium increased the number of lateral tips developed.
- Increases in lateral root formation may have implications in nutrient uptake, competitive ability, and overall reproductive success in these mutant lines.

## Citations


O'Malley R.C. and Ecker J.R., 2010. Linking genotype to phenotype using the Arabidopsis unimutant collection. *The Plant Journal*, 61(6): 928-940.

Valentine, M.E., Wolyniak, M.J., and Rutter, M.T., 2012. Extensive Phenotypic Variation among Allelic T-DNA Inserts in *Arabidopsis thaliana*. *PLOS One,* 7(9): e44981.

Williamson et al., 2001. Phosphate Availability Regulates Root System Architecture in Arabidopsis. *Plant Physiology*, 126(2): 875-882.



**Acknowledgments** : Special thanks to Matt Rutter, Allan Strand, Elsa Cousins, Bravada Hill, Liv Stewart, and all of the CofC unPAK team for their help.

IOS1146977
IOS1052262
IOS1355106