Knocking It Out of the Park: Arabidopsis Mutants Don't Display a Decrease in Fitness

Emma Bergh, Jack Poyle, Jun Takai, Nia Daids, Karsten Jurkiewicz and Angie Roles, Oberlin College

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Abstract

The genome sequence of the model plant *Arabidopsis thaliana* has been available for fifteen years, yet gene function is known for only about 10% of its 26,800 genes. At a broader level, gene function may be investigated by generating a mutant that has the gene of interest 'knocked out' via a tDNA insertion, so the mutant lacks the gene product. The mutant is then grown under various conditions and phenotyped for traits such as life history or morphology. As part of unPAK (Undergraduate Phenotyping of Arabidopsis Knockouts), we have been investigating the phenotypic effects of single-gene knockouts of the Columbia genotype of *A. thaliana*. Our set of mutants consists of 122 knockout mutant genotypes and 11 phytometers, serving as controls. These mutants have been grown twice, once with a heat stress and once under control environmental conditions. Results showed few differences in plant performance between the mutants as a group and the control phenotypes for both experimental runs. The mutant phenotypes had a wider range of variation, but were not on average, less 'fit' than the control phenotypes. To identify potential gene effects, we took a closer look at mutant genotypes with phenotypes that differed significantly from the control by researching previously documented information on the mutated gene using The Arabidopsis Information Resource (TAIR, www.arabidopsis.org). This allows the phenotype to be associated with a specific locus where the genes were altered, and we can see what changes when the gene is non-functioning. Issues with regulating temperature and controlling fungus growth in our experimental setup merit more trials so more can be understood about this vast genome.

Methods

Planting Design. We grew a total of 122 mutant genotypes and 11 phytometers as part of this set, with 6 replicates of each mutant genotype and 12 replicates of each phytometer. Plants were grown in trays, 12 trays per experiment with 72 pots per tray. Each tray contained one replicate of each phytometer and 61 mutants. Mutant genotypes were assigned randomly to each tray such that a single genotype was only represented once per tray. This process was repeated twice, with one set being grown in summer 2015, representing the heat stress treatment and one being grown in fall 2015, representing the control.

Growth Conditions. Prior to being sown to soil, the seeds underwent cold-treatment to increase standardization of germination time. Seeds were placed onto moist filter paper in the dark at 4℃ for one week. Seeds were planted into moist soil and moved into a controlled environment room for the rest of the experiment. Two seeds were planted in each pot. Two weeks after planting, each pot was thinned to contain a single seedling. Plants experienced 16h light and 8 h dark at 22. Trays were watered as needed. Plants were harvested for additional phenotyping after senescence.

Heat Stress Treatment. During the 5th week of the summer experiment, just as plants reached the stage of bolting, accidental heat stress occurred due to a malfunction in the air circulation system. Without air circulation, temperature increased and decreased dynamically in accord with the activation and deactivation of the light sources. Plants were removed from the growth chamber upon discovery of the problem and were returned after repair, about 7 hours later. The heat stress lasted a total of 14.5 hours, with a max temp of 43℃, which is about 20℃ higher than normal (Figure 1). Plants were closely monitored for stress responses in the week following the heat stress.

Phenotypes Recorded. Plant phenotypes were observed throughout the life cycle and the following traits were recorded:

- Germination: For two weeks post-planting, recorded date when cotyledons were visible.
- Bolting: When the plant's inflorescence reached at least 5 mm, the diameter of the rosette (mm) and the date were recorded.
- Flowering: We recorded the date that open flowers were first observed.
- Number of branches - including basal, main stem, and branches of branches.
- Plant height (cm) from the rosette to the terminal bud of the main stem.
- Number of good fruits (fruits capable of forming normal seed).
- Length (mm) of 3 representative good fruit, chosen haphazardly
- Number of aborted fruits.

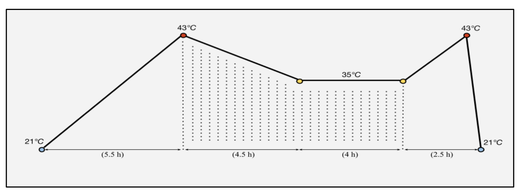


Figure 1. Diagram describing the change in temperature during the course of the heat stress. Lights were off during the dotted area of the graph.

Overall Results

Intuitively, we might predict that knocking out an entire gene would have a dramatic and visibly perceptible effect on the organism. However, average trait values of individuals with a single knockout mutation are not significantly different than average trait values of the individuals from the non-mutated Columbia genotype (Table 1). However, for the summer dataset, there were significant differences in trait values when our mutants were compared to the 10 natural genotypes we grew. We found that plant height was greater, on average, in the knockouts comared to the controls. In addition, rosette diameter at bolting was significantly larger in the natural genotypes versus the knockout mutants (but neither group was different in size from Columbia).

Table 1. Sample sizes, means and standard deviations of phenotypes for three groups of genotypes. "Columbia" is the non-altered genome of *A. thaliana,* "Natural" represents the 10 other phytometer genotypes and "Knockout" represents the altered genotypes.

	Summer			Fall		
Group[1]	N	Mean	St.Dev	N	Mean	St.Dev
Height						
Columbia	12	13.33^{a,b}	4.68	12	1.92	1.36
Knockout	718	13.59^{a}	4.34	677	2.71	3.44
Natural	115	11.04^{b}	6.04	68	2.37	3.97
Fruits						
Columbia	12	6.92	4.06	12	1.00	0.74
Knockout	718	7.50	6.12	677	2.12	7.21
Natural	115	7.4	6.13	68	2.03	5.77
Rosette Diameter						
Columbia	12	20.46^{a,b}	5.73	12	5.36	1.97
Knockout	716	19.25^{a}	5.74	674	6.74	4.84
Natural	116	22.92^{b}	9.24	66	7.00	5.16
Total Branches						
Columbia	12	0.17	0.39	12	0.00	0.00
Knockout	726	0.33	1.20	730	0.39	1.46
Natural	120	0.40	1.24	120	0.30	0.98

[1]For a given trait within a Set, group means that do not share a letter are significantly different with $P<0.05$.

We examined relationships of several plant peformance traits with plant height: rosette size at bolting, fruit number, and branch number (Figure 2). All three displayed similar patterns: performance is positively correlated with height. We noticed that in each graph, there is a clear cluster of knockout mutants near the Columbia genotype, with the natural genotypes generally displaying similar or greater range of phenotypic values compared to the knockout mutants.

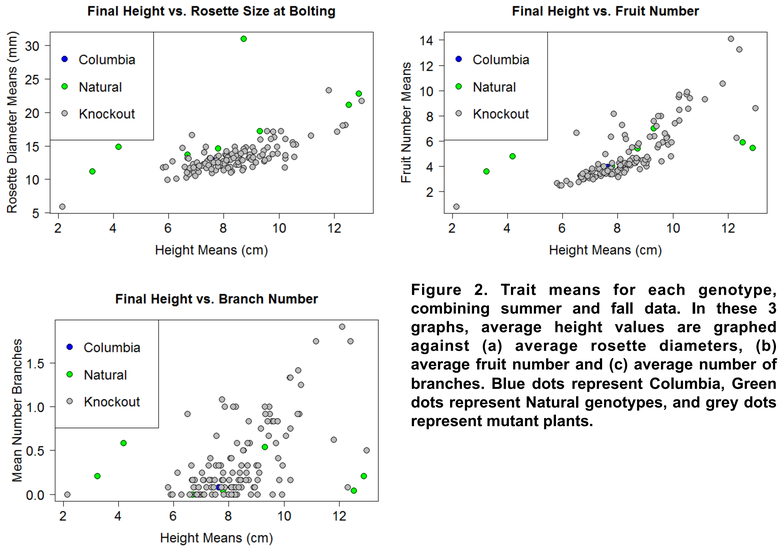
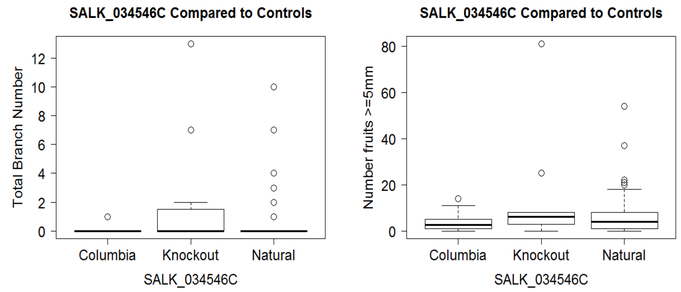


Figure 2. Trait means for each genotype, combining summer and fall data. In these 3 graphs, average height values are graphed against (a) average rosette diameters, (b) average fruit number and (c) average number of branches. Blue dots represent Columbia, Green dots represent Natural genotypes, and grey dots represent mutant plants.

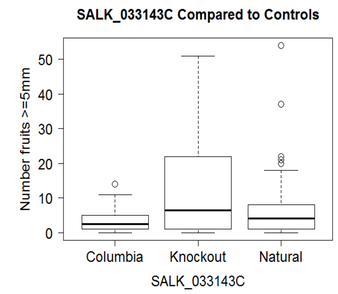
In both summer and fall, our experiments experienced unexpected conditions: heat stress in the summer and a factor causing stunted plant growth in the fall (possibly fungus). Thus, differences between the summer and fall plantings cannot be attributed to presence/absence of the heat stress.

Looking at Individual Genotypes

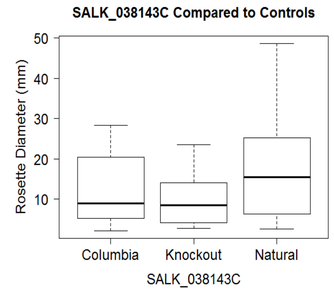
Using our combined data set of summer and fall, we analyzed variation between the different genotype groups with univariate ANOVAs including group (Columbia, Natural, or Knockout), time (summer or fall), and accession as explanatory variables. For the four traits with significant differences among groups, we performed pairwise comparisons (Tukey HSD tests): fruit number, branch number, height, and rosette diameter. Individual knockout accessions (genotypes) that differed significantly from Columbia or the Natural Genotypes by the Tukey test were researched via TAIR to explore potential gene effects and functions.



SALK_034546C The total branch number was marginally significantly greater versus a control (P=0.099), and fruit number was significantly greater versus a control (P=0.047). The knockout mutation was done at the AT2G205 locus, which is known to produce a protein that is associated with the mitochondrial respiratory chain complex I. The protein is located in cell, mitochondrial and vacuole membranes. With a greater fruit number and more branches on average, this knockout appeared to be beneficial to the organism.



SALK_033143C The number of fruits in this knockout was significantly greater versus a control (P=0.027). This mutant was knocked out at the locus AT3G41768, which is involved in translation and the cytosolic small ribosomal subunit.



SALK_038143C The rosette diameter of this line was marginally significantly smaller versus a natural phenotype control (P=0.102). The knockout mutation in this line occurred in the AT5G635301 locus, which has been proven to be associated with metal ion binding and transport.

Acknowledgements

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Conclusions and Further Work

Under both heat stress and fungal conditions, knockout mutants do not, on average, perform worse than the control plants. This apparent robustness in survivorship in the face of gene knockout suggests that many individual genes may not be critical to normal organismal function. For example, widespread gene redundancy may act as insurance against silencing mutations, while polygenic and epistatic phenotypes may be buffered as well. Further genome analysis could be used to understand how many genes can continue to work while some are not performing their normal functions, and see how these plants grow under other stressful conditions. The technique of identifying lines with significant differences from the control has proved useful to relate phenotype to specific loci on a basic level, and will allow for future studies. At Oberlin, we are currently running a control trial for a second set of mutants that were grown in summer 2015 and experienced the heat stress, along with the same controls. We hope that this will serve as the non-stress control for these plants.

Picture 1. Set 2, *Arabidopsis thaliana* at days 7 and 11.