Variability Across Multiple Growth Chamber Experiments Exceeds Variability Between Greenhouse and Growth Chamber Environments

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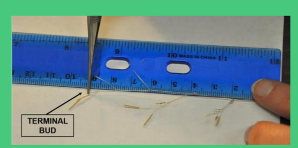
Introduction

- The undergraduate phenotyping of Arabidopsis knockout project (unPAK) seeks to evaluate the phenotypic effect of single gene knockouts from *Arabidopsis thaliana's* genome.
- Our focus was to quantify phenotypes from the greenhouse experiment and compare the resulting phenotypic effect amongst past growth chamber experiments.
- We hypothesized that growing plants in the Barnard greenhouse could elicit different phenotypic traits than Barnard unPAK's previous and highly standardized growth chamber experiments. Specifically, we looked for considerably higher or lower mean values as well as for greater variability for our focal traits considering that greenhouse conditions are a departure from chambers and are generally assumed to provide less of a controlled environment.
- Knockouts are grown side by side with phytometers (wild-types) to identify outlying phenotypes.

Focal Traits

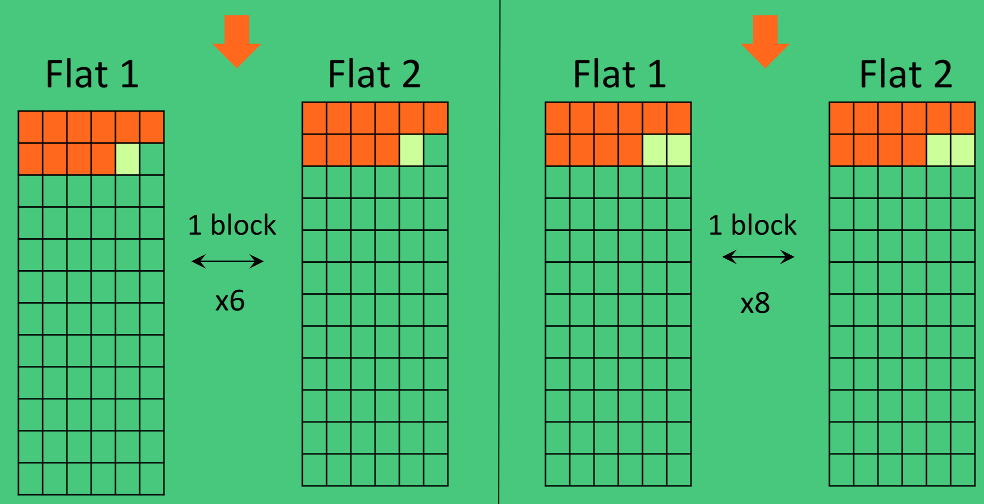
To quantify the phenotypes, we measured specific traits that are essential to the plant's survivorship

Height: Measured from rosette to terminal bud
Rosette Diameter: Measured across rosette at widest point when inflorescence is taller than 5mm
Bolt Days: Bolting recorded when inflorescence is taller than 5mm
Fruit Number: Each silique larger than 5mm are counted as a full fruit
Fruit Length: Measured siliques at different points along the main stem




Experimental Design

Growth Chamber	Greenhouse
To date, 5 Experiments	First effort summer 2016

Growth Chamber	Greenhouse
Total = 20 Phytometers	Total = 20 Phytometers
2 Columbia	4 Columbia
122 knockouts	120 knockouts

Planting: The seeds are placed on filter paper inside a petri dish and then placed into the cold room to simulate a winter environment. When removed, the 72 genotypes are randomized across each flat and then moved either into the green house or the growth chamber.
Maintenance: The flats are watered twice a week and once germination begins, the flats are checked daily for bolting and measured for rosette diameter.
Harvesting: At the time of harvest, the set of fitness-related traits are measured including height, fruit number, fruit length as well as aborted fruits and the plants are then collected for future reference.

Results

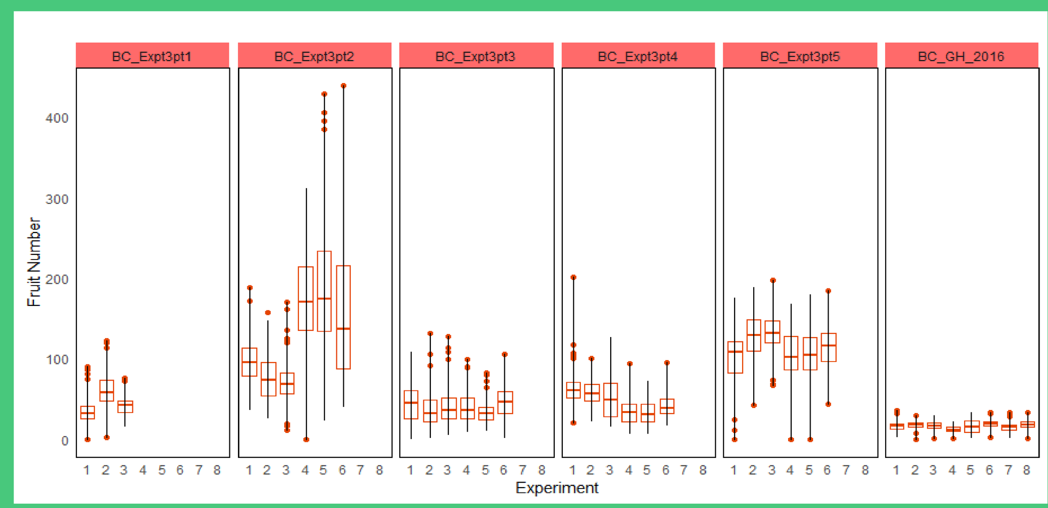


Figure 1. Number of fruits by experiment and by block . There was a total of 5 growth chamber experiments (each with 6 blocks and one greenhouse experiment (8 blocks). **Fruit number ranged from 50-250 in the growth chambers whereas the greenhouse ranged from 10-30 fruits and also showed lower variability. This trait was exceptional in supporting our hypothesis.**

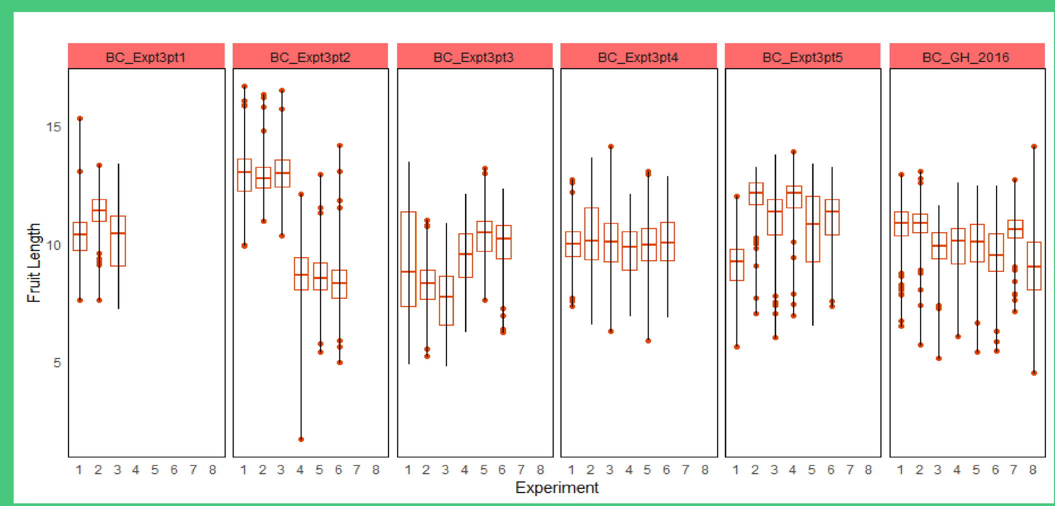


Figure 2. Fruit Length by experiment (6 experiments) and by block (6 growth chamber blocks. and 8 greenhouse blocks.). **The green house experiment doesn't necessarily differ from experiments in chambers either in mean values or in variability for this trait. For this trait, BC_Expt3pt2 displays high variation which doesn't suggest that difference in mean values is due to a change of environment. This and the rest of the traits except for fruit number did not support our hypothesis.**

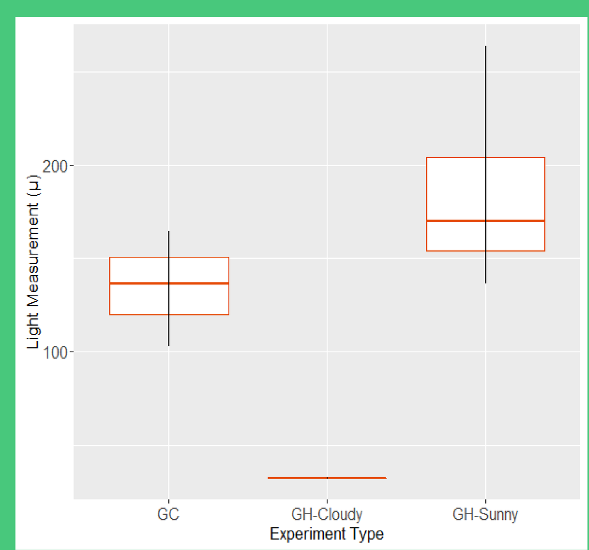


Figure 3. Light Measurement by experiment. **Compared to the growth chamber, the greenhouse light measurements differ in mean values between a sunny and a cloudy day. However, between the growth chamber and the greenhouse experiments the variability for this environmental factor doesn't necessarily differ.**

Environmental Conditions

For genotypes that are examined for phenotypic performance, the one that grows best in one particular year, may show only average performance in the next due to annual differences in environmental conditions. Specifically, there are five important abiotic factors that are crucial for the performance of the plant.

1) Light quantity: Light distribution is an important factor that impacts plant growth specially in growth chambers where light intensity tends to drop by 20-50% close to the walls. Also, the lack of constant sunlight that the plants are receiving in the greenhouse can be a cause of variation in experimental results.
2) CO_2concentration: In most growth chambers, CO_2 concentrations are not controlled. For those that have a large biomass, the CO_2 concentration increases during the night and decreases during the day. In addition, if there is high human traffic in the chambers or in the greenhouse during the day it can lead to an unwanted CO_2 enriched environment altering the plant's growth.
3) Air Humidity: As humidity increases in either the greenhouse or the growth chambers due to a lack of wind, high radiation or other factors, it could cause a decrease of evapotranspiration and therefore a decrease in the overall performance of the plant.
4) Temperature: In the green house, solar radiation can affect the temperature of the pots, specially those that are colored black causing an increase in evaporative demand and sometimes causing the plants to die. In addition, when the plants reach their maximum height in the growth chambers, radiative heating is emanated from the bulbs causing the leaves at the very top to either overheat or sometimes grow quicker.
5) Confounding Factors: Other factors affecting plant's growth can be contamination from paint or materials used to make the growth chambers, ozone concentrations, and a common one, pest damage.

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References

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