Identifying Potential Biomarkers For Non-Infectious Bud Failure

IAB MEETING 11/19/24

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Overview

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Project Description: Goals

The overall all goal of this project was to identify potential biomarkers for the purposes of screening for Non-Infectious Bud Failure (NBF) in Almond.

The project consisted of two primary objectives:

1. Using previously identified differentially methylated regions, test candidate genes against bud failure and non-bud failure trees using DNA methylation markers.
2. Analyze gene expression at candidate gene sites using quantitative polymerase chain reaction (qPCR) to determine if there is variable expression at gene sites corresponding to bud failure expression.

Project Description: Experimental Design

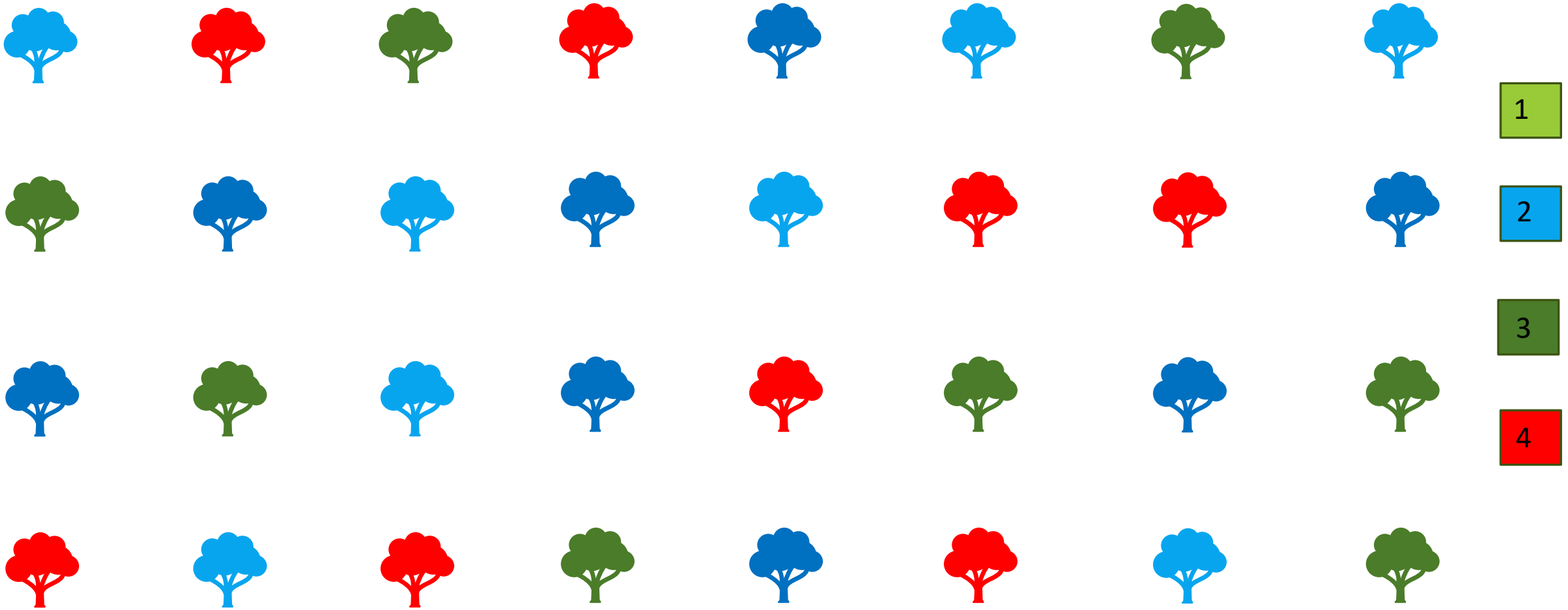
We are trying to detect genetic variation between clones of Carmel expressing different levels of bud failure

- Trees score on a 1-4 scale
  - 1 being low NBF (0%-25% canopy affected)
  - 2 being medium-low NBF (25%-50% canopy affected)
  - 3 being medium-high NBF (50%-75% canopy affected)
  - 4 being high NBF (75%-100%)
- 32 Trees sampled randomly in commercial orchard
  - 8 dereplicates of each NBF level taken

Two methods to analyze samples:

- Enzymatic methylseq to produce DNA methylation data.
- 3`TAG RNA Seq (Revised from original project objective) to produce gene expression data.

Project Description: Experimental Design



Project Description: Experimental Design

1: Little to no NBF present. No Major reduction in yield.

2: Some NBF present on up canopy shoots. Minor reduction in yield.

3: Significant NBF present on major scaffolds. varying reduction in yield.

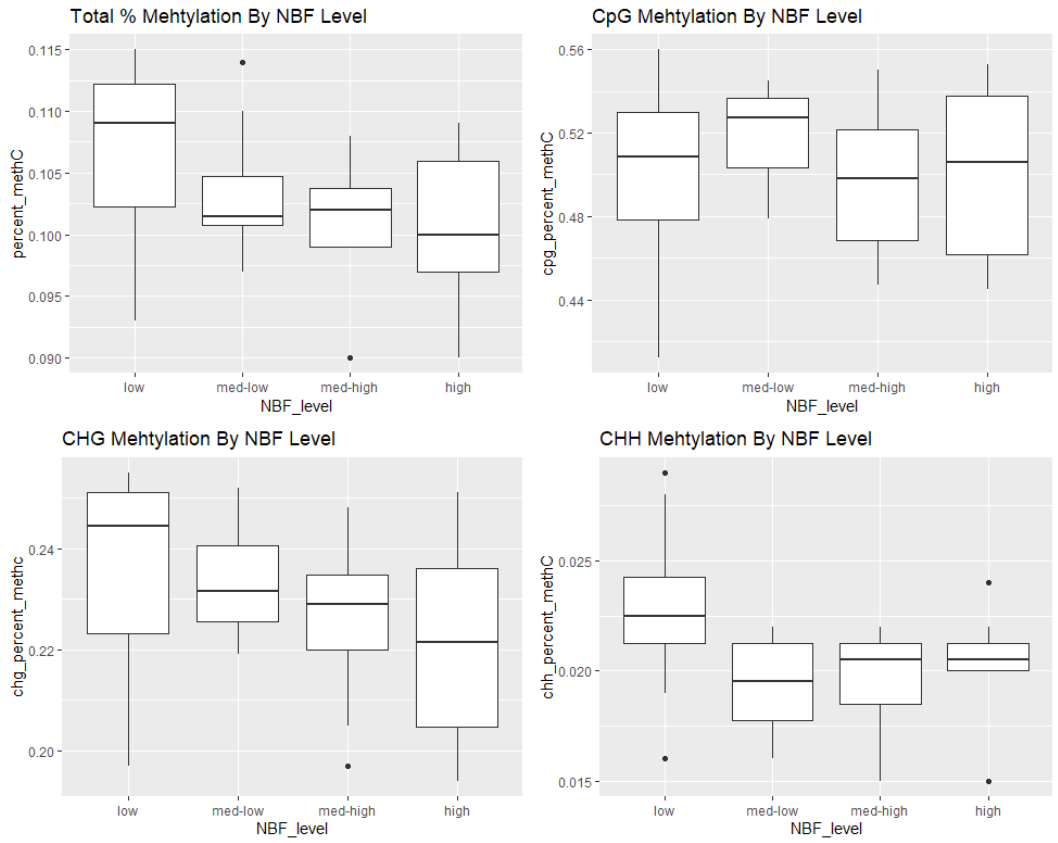
4: NBF is severe throughout tree. Heavy reduction in yield.

Results: Global Methylation

Broad Trends: Total cytosine methylation was measured at each Context (CpG, CHG, CHH)

Results: Trends suggest an overall <u>decrease</u> in methylation as the rate of bud failure increases.

However, after performing an ANOVA test to determine significance of differences between levels, no significant differences were found.



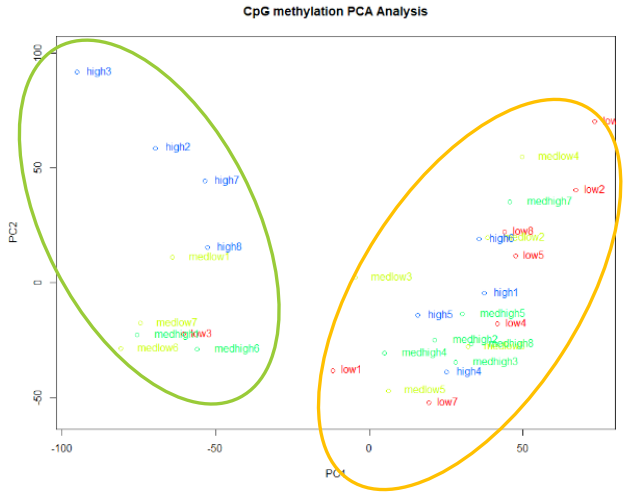
Results: Differential Methylation

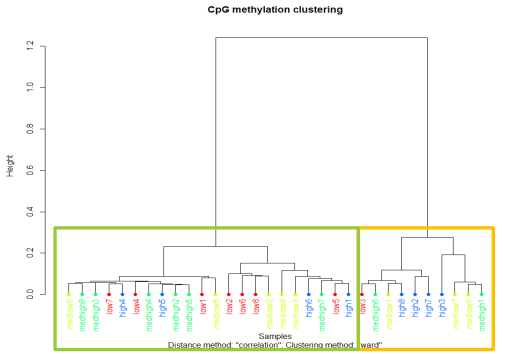
Finer Trends: Measured differences in methylomes between individual samples with varying levels of bud failure.

Principal Component Analysis (PCA) and a Dendrogram were made based on the methylomes of samples were made. These show relationships between samples.

Results: Clustering between sample is occurring along two primary groups, and samples with the same level of NBF tend to cluster together.

However, outliers make hard conclusions difficult.





Results: Differential Methylation

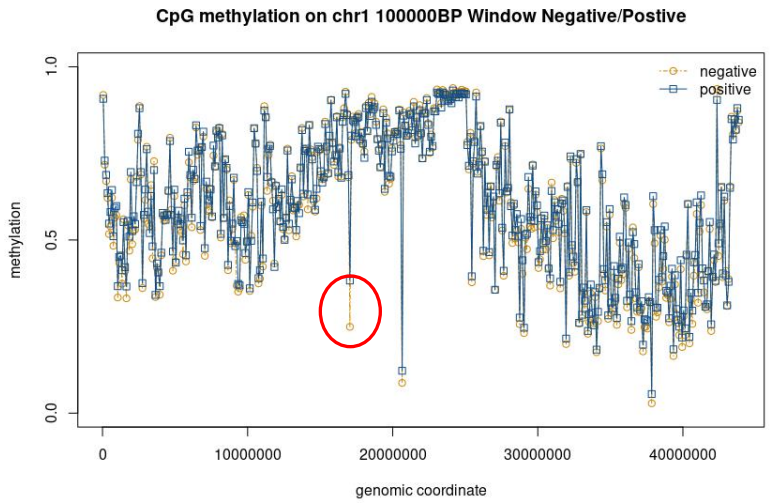
Subdivide Groups: Since we started to see larger trends, we divided samples into a positive(Levels 2, 3 and 4) versus negative group(Level 1). We then conducted analysis of differential expression based on these groups

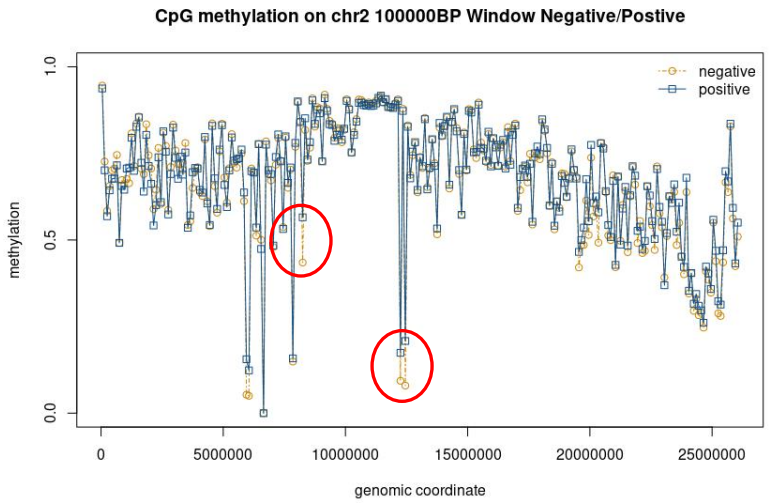
Created low-resolution methylation profiles:

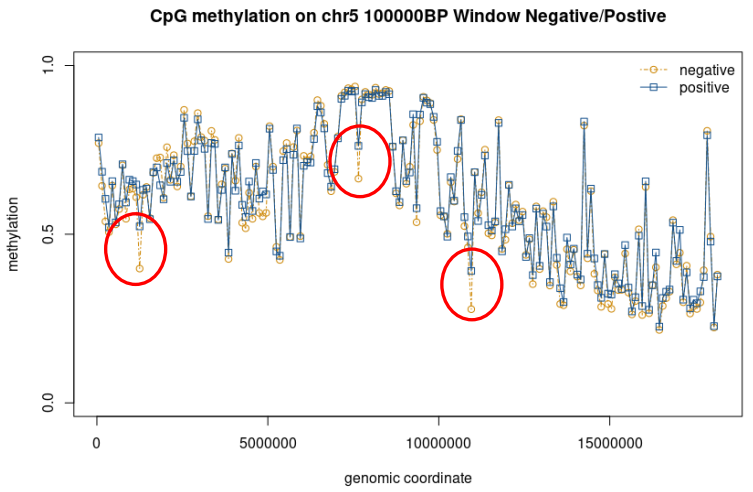
- Cut samples into 100,000 base-pair windows
- Compared windows of positive versus negative to samples to identify regions with differential methylation.
- One profile for each chromosome.

Results: We found areas of differential methylation on Chr1, Chr 2 and Chr 5.

Results: Differential Methylation







Results: Differential Methylation

Gene Specific Methylation: Using our low resolution profiles as guides, we used annotations of known genes to determine differentially methylated regions(DMRs) between the positive and negative NBF groups.

Results: In total, we found 20 DMRs between the negative and positive groups. Primarily, these occurred in exogenic regions, but including some genomic and protein coding regions.

Chromosome #	Length	Region Type	Gene Tag	Methylation in Positive Group
chr001	94	exon	L3X38_005079	gain
chr002	62	exon	L3X38_013331	gain
chr002	297	gene	L3X38_012770	loss
chr002	297	mRNA	L3X38_012770	loss
chr002	297	exon	L3X38_012770	loss
chr003	95	exon	L3X38_015741	gain
chr003	44	exon	L3X38_016203	gain
chr004	153	exon	L3X38_021823	loss
chr005	1777	gene	L3X38_026510	gain
chr005	1777	mRNA	L3X38_026510	gain
chr005	138	exon	L3X38_026517	loss
chr005	176	exon	L3X38_028362	gain
chr006	82	exon	L3X38_032567	gain
chr007	306	gene	L3X38_038734	gain
chr007	306	mRNA	L3X38_038734	gain
chr007	306	exon	L3X38_038734	gain
chr008	183	exon	L3X38_042585	gain
chr008	390	gene	L3X38_042980	gain
chr008	390	mRNA	L3X38_042980	gain
chr008	390	exon	L3X38_042980	gain

Results: Gene Expression

Differential Gene Expression Analysis: To examine how genes may differ between levels of NBF, we looked as gene expression data, or “how much” of a certain gene is represented in each sample.

These samples were taken from genetically identical trees from the same tissue type. The only difference between them is overall NBF severity. When compared to the lowest NBF samples:

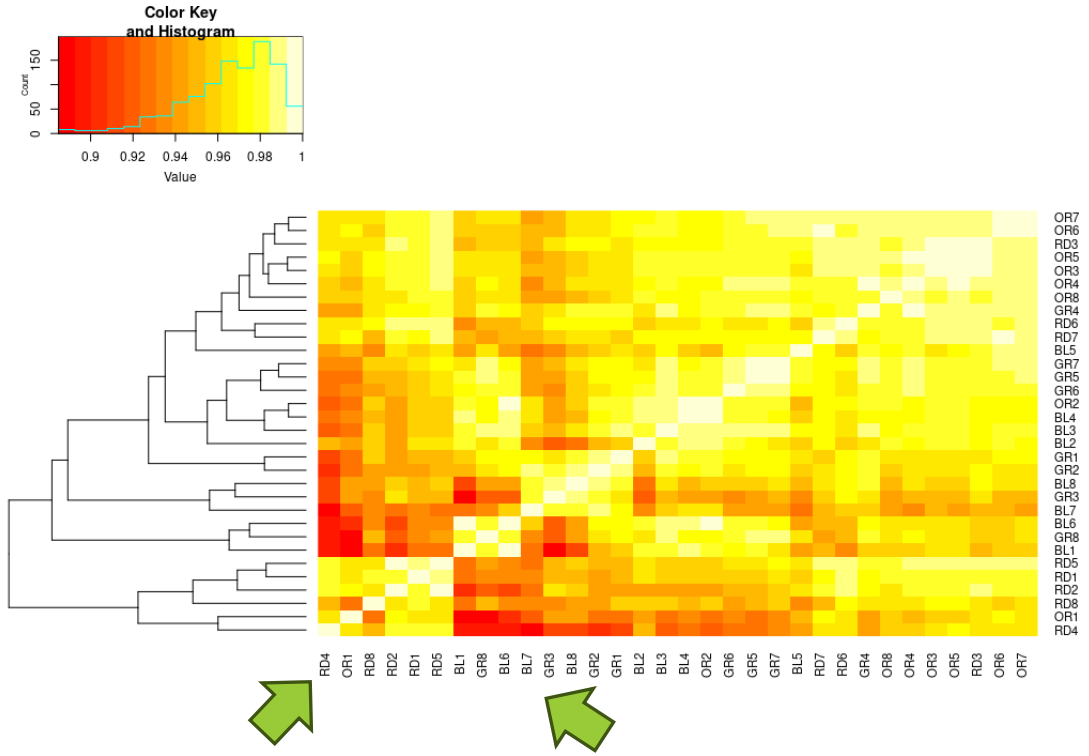
- 456 significant differentially expressed genes
  - 254 upregulated
  - 202 down regulated

Results: Gene Expression

Correlation Of Expression: We constructed a heat map to show the correlation between the expression levels of the samples.

- The lighter the box, the more similar it is. The darker the box, the more dissimilar.
- Samples have a correspond tag
  - RD is low
  - OR is medium high
  - GR is medium low
  - BL is low

Results: Correlation is strongest between samples with similar levels of NBF.

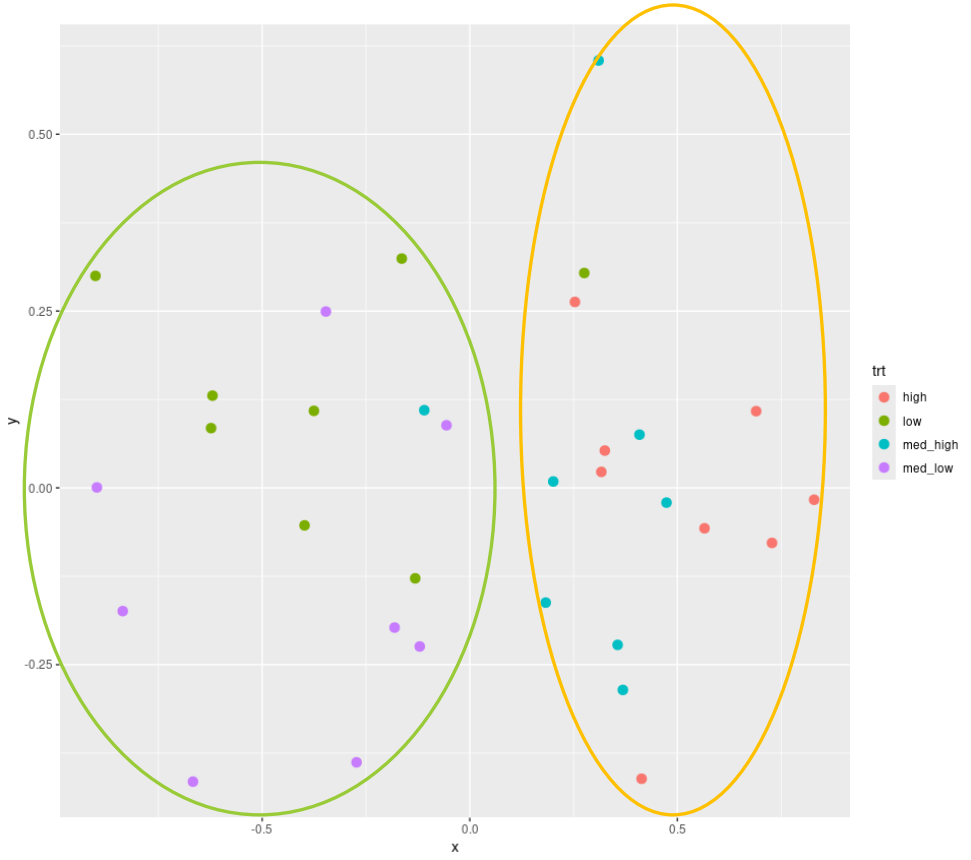


Results: Gene Expression

Relationship of Samples: In order to measure the relationship between the samples and measure the level of similarity, we constructed a multi-dimensional scaling (MDS) plot. The closer the points are to one another, the more similar they are.

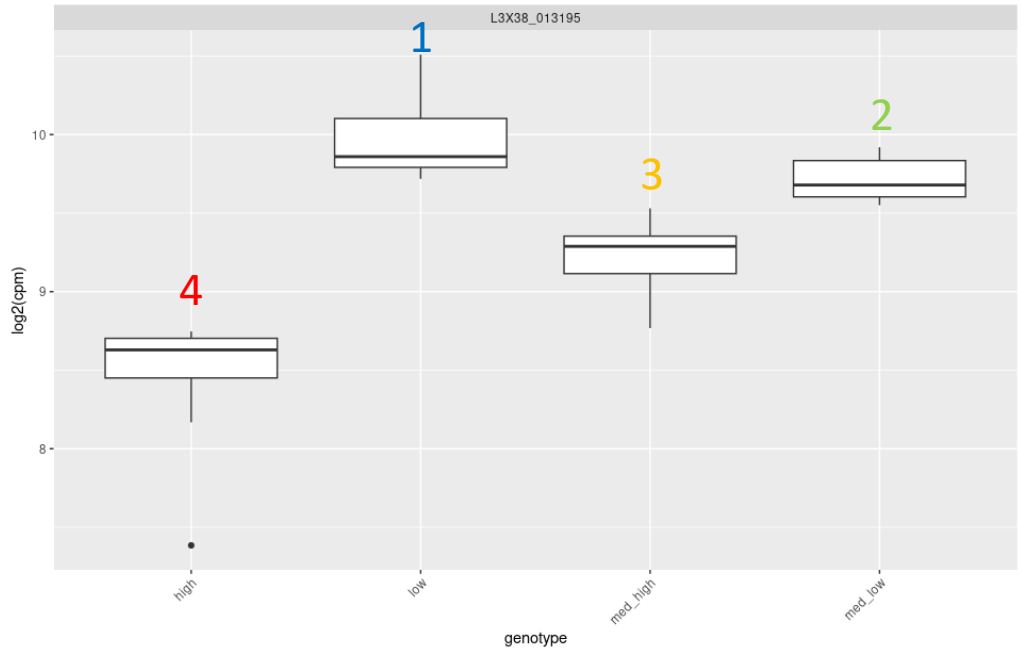
Results: The samples cluster in two groups: one with little to no bud failure, and ones with some or high bud failure.

However, the intermediate phenotypes add noise and blue distinction. Some points also show some overlap.



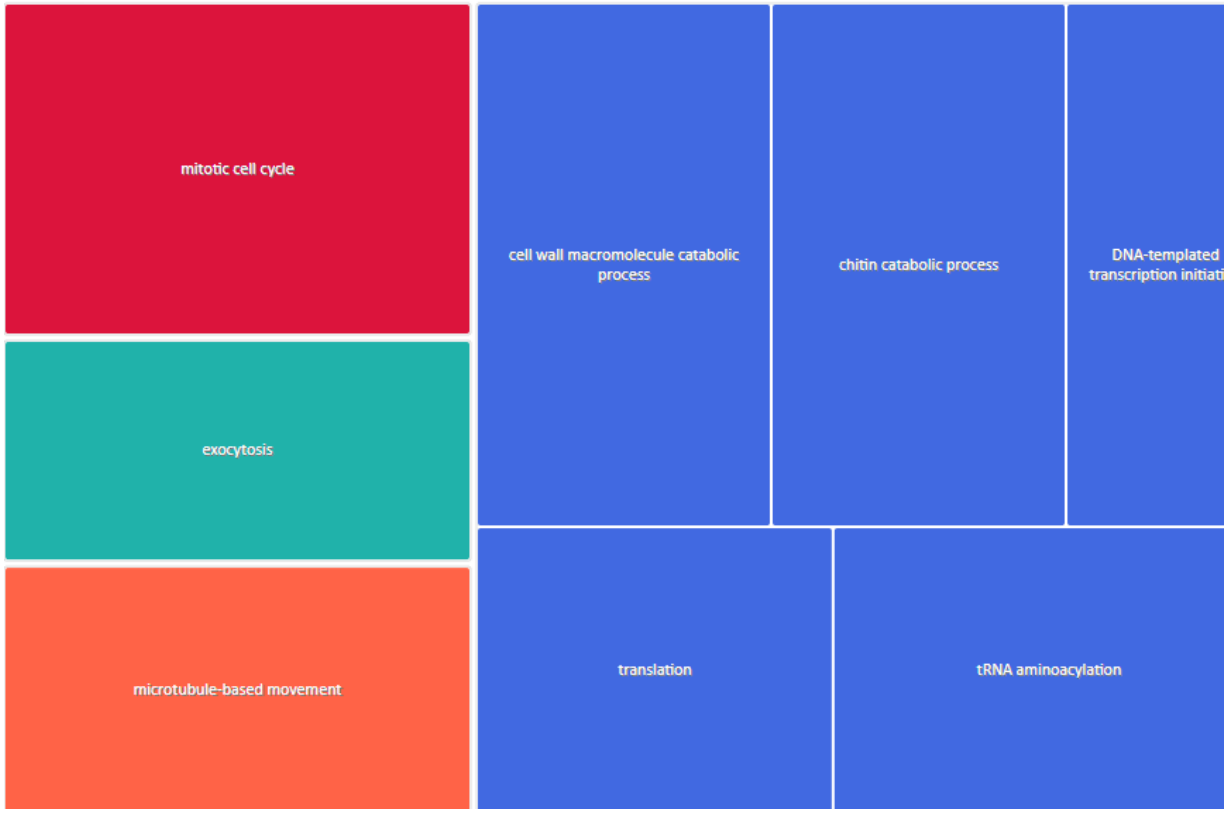
Results: Gene Expression

Individual Gene Expression Levels: We then examined how expression may change at individual gene sites for each level of NBF.
Results: Expression at certain significant gene sites changes as the severity of bud failure increases.
A pattern across all genes is still unclear. These are a snapshot in time, and don't describe a progression.



Results: Gene Expression

Gene Ontology: While we have data on individual gene expression, it is unclear what those genes do. We conducted a gene-ontology analysis, which uses similarities across all plant species to identify motifs and other similar gene functions with annotations.
Results: We found that the gene ontologies of many of the differentially expressed gene relate to cell division and plant-cell wall construction. Additionally, we found gene ontology terms related to the regulation of DNA methyltransferase, which controls DNA methylation.
These are primarily speculative.



Next Steps/Future Studies

- Explore Relationship between methylation and gene expression.
- Identify roles of individual genes.
- Examine differential methylation and gene expression with previous published studies.
- Resample trees to mark change over time:
  - Whether level of bud failure has changed.
  - Whether expression and methylation/expression has changed in corresponding genes.
- Long-term prediction study